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5308

Pathology of Experimental Vaccinal and Rabies Encephalitis.

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One of the primary infections[1] of the central nervous system of man, is termed post-vaccinal because it is most frequently found after vaccination against small pox. Yet the identical pathological changes have been described after vaccination against rabies,[2] complicating measles, small pox, varicella and upper respiratory infections. This, of course, does not necessarily mean that a single virus is the responsible agent. The reaction of the nervous system is more or less stereotyped and the same reaction may occur in several diseases such as is the case in Heine-Medin's disease, epidemic encephalitis, and true rabies.

In the human, post-vaccinal type of encephalomyelitis there occur disseminated foci of perivascular demyelinization, especially about the veins, coalescing and extensions of the lesions to destroy the whole nerve fiber and to form true softenings composed of intra and extracellular fat. Very few or frequently no lymphocytes are found in the perivascular spaces, and meningeal exudates occur only secondary to the softenings.

[1] Grinker, R., and Bassoe, P., *Transactions Am. Neur. Assn.*, 1930.

[2] Bassoe, P., and Grinker, R., *Arch. Neur. and Psych.*, 1930, **23**, 1280.

Experiments to discover whether the encephalomyelitis associated with vaccination was due to the vaccine virus have given conflicting results. (McIntosh and Scarff[3] and Aldershoff,[4] Eckstein and Herzberg,[5] McIntosh,[6] Hurst and Fairbrother,[7] Thompson,[8] Pette.[9]) Many investigators contend that vaccination in the human against small pox or rabies, or the exanthematous diseases does not produce encephalitis by the introduction of a specific virus but by stimulating a latent virus to activity.

It was attempted to determine whether in the experimental animal, vaccinia encephalitis could be produced, and whether it resembled the human type. Furthermore attenuated rabies was introduced into experimental animals to determine its pathological characteristics, to compare it with vaccinia encephalitis and human post-vaccinal (rabies) encephalitis. Rabies was used particularly because it could be identified by the presence of negri bodies.

Intra-cerebral injections of an ordinary vial of calf lymph virus were made. In one guinea pig death occurred in 4 days from a severe meningo-encephalitis. One guinea pig showed no clinical symptoms but on autopsy a meningo-encephalitis was found. Further transmission of this encephalitis from the sources was unsuccessful. Intra-cerebral vaccinia injections into rabbits produced no symptoms and anatomically showed no encephalitis. Emulsions of these brains caused no encephalitis when injected intracerebrally in other animals. Intra-testicular injection of vaccine virus, however, produced in one out of 3 rabbits a fatal meningo-encephalitis which could be carried on to other rabbits by intracerebral inoculation of an emulsion of its brain. Subcutaneous inoculation of vaccine virus was unsuccessful but corneal inoculation produced a high-grade encephalitis.

Commercial rabies vaccine was used as the source of attenuated "fixed" virus. One-half cc. was injected intracerebrally into a dog and in 7 days the animal was dead after showing the typical clinical picture of rabies. An emulsion of the animal's brain was passed through several series of rabbits until by storage in glycerine and passage the time required to kill a rabbit after intracerebral inoculation was only 4 days.

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[3] McIntosh, J., and Scarff, R. W., *J. Path. and Bact.*, 1930, **33**, 483.

[4] Aldershoff, H., *Acta. Path. and Microbiol. Scand.*, 1930, **3**, 9.

[5] Eckstein, A., and Herzberg, K., *Deutsche med. Woch.*, 1930, **57**, 264.

[6] McIntosh, J., *Brit. Med. J.*, 1928, **2**, 334.

[7] Hurst, E. W., and Fairbrother, R. W., *J. Path. and Bact.*, 1930, **33**, 463.

[8] Thompson, R., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 559.

[9] Pette, H., *Munch. Med. Wochenschr.*, 1928, **75**, 207.

The brain and spinal cords were fixed in formalin. Sections stained by special methods to demonstrate fat, microglia, myelin sheaths, axones and the usual hematoxylin-eosin stain was also used. In no single specimen was there any evidence of demyelinization, axis cylinder destruction or accumulation of fat. Thus in no manner did the vaccine virus or attenuated rabies vaccine produce a pathological change similar to the post-vaccinal encephalitis in man.

Both series showed a severe meningo-encephalitis. In the vaccinia experiments, the meningeal exudate consisted of many leucocytes mixed with histocytes while the intracerebral vessels were infiltrated with moderate numbers of lymphocytes and the adventitial cells were increased. The rabies group showed an intense lymphocytic meningeal exudate and perivascular infiltration. Negri bodies were found in every case. There was a diffuse ganglion cell degeneration and increase in microglia. Thus in both types of infection a diffuse meningo-encephalitis occurred, varying only in the predominating type of cell and intensity of reaction.

These experiments show that, in the experimental animal at least, attenuated rabies and vaccinia do not produce the pathological changes found in human post-vaccinal (cow pox and rabies) encephalitis. These findings would seem to suggest that the various types of vaccination and exanthematous diseases in man might act as a stimulant to a latent infection rather than by introducing a specific virus.

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New Technic for Roentgenographic Study of Renal Vessels.*

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Ralph Graham[1] applied the method of Hill[2] to the injection of the vascular tree in the human kidney. The method, involving the injection of a 25% bismuth oxychloride suspension in 10% acacia, is applicable to postmortem material. In such kidneys it demon-

* The present investigation was aided by a grant from the Josiah Macey, Jr., Foundation.

1 Hill, E., *Bull. Johns Hopkins Hosp.*, 1929, **44**, 248.

2 Graham, R., *Am. J. Path.*, 1928, **4**, 17.

strates pictorially the blood vessels in x-ray photographs in a manner superior to the barium injections previously used. Since we wished to demonstrate the changes induced by the action of various agents on the renal vessels during life we set out to adapt the method of Hill and Graham to this end.

It is apparent that such solid particles are rapidly removed from the blood stream upon injection, and so in order to obtain them in sufficient concentration in a particular organ to demonstrate the vessels roentgenologically, the opaque substance must be injected directly into or close to the origin of the artery supplying the organ. As we were interested in comparing the relative appearance of the vascular beds in the 2 kidneys, the aorta close to the origin of the renal arteries was chosen as the site of injection.

Dogs were used throughout. Under general anesthesia (Nembutal—Abbott) a left paravertebral incision was made extending from a point 2 cm. above the costal margin caudad for a distance of 7-10 cm. The last rib was removed without opening the pleural cavity and the aorta was approached extraperitoneally and exposed between the crura of the diaphragm. It was also exposed below the origin of the renal vessels at which point it was clamped. The upper segment was lifted into view upon the finger and 50 cc. of a 25% suspension of bismuth oxychloride injected into the aorta through a large bore needle. (bismuth oxychloride (Cosmetic) 25 gm.——water q.s. ad 100 cc.) The puncture point of injection in the wall of the aorta was clamped and after 5 to 10 minutes the kidneys were removed and the animal killed.

The kidneys were hemisected and flat x-ray pictures were made, using a low voltage and low milliamperage with the 3 to 5 second exposure.

In a few animals injection was made into the aorta, below the origin of the renal vessels, with or without intermittent occlusion of the aorta above the level of the renal arteries. The results were somewhat less satisfactory than with the method described above.

A simple suspension of bismuth, without the use of acacia, was found to be most satisfactory in this work and, as was pointed out by Hill and Graham, the most finely divided form of bismuth must be used to obtain delineation of the small vessels.

Postmortem injection of the kidneys of dogs and rabbits was found to be unsatisfactory, although in dogs even glomerular injection can be obtained with India ink.

The arterial system was visualized on the x-ray plate to and including the fine interlobular vessels. (Fig. 1.) In the normal speci-

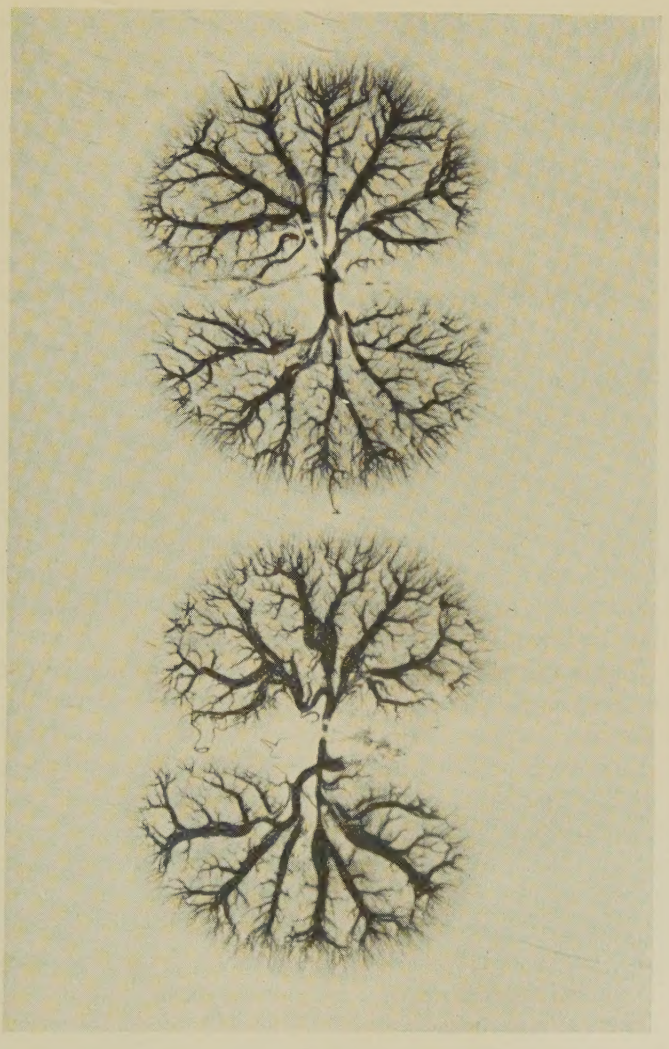

FIG. 1.
Injected kidneys, normal young dog. Note the uniformity of extent and distribution of the arcuate and interlobular vessels.

mens injection of the fine vessels was uniform throughout the kidney. The projection of the interlobular vessels towards the outer margin of the cortex was also uniform. As was pointed out above, glomerular injection was not noted in the x-ray pictures, and was very irregular in the histological sections made from such injected specimens.

One old dog, whose kidneys were grossly scarred and on section showed marked arteriolar alterations, was injected. The picture

obtained is striking in several respects and simulates those described by Graham in human cases of arteriolar-sclerosis. First the roughly beaded appearance of the vessels and, secondly the incomplete and patchy injection of the vascular bed.

5310

Studies in Renal Denervation. I. Roentgenographic Demonstration of Vascular Alteration.*

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The physiological and anatomical changes induced by denervation of the kidney have been carefully studied by Seres,[1] Ellinger and Hirth,[2] Bieter,[3] Bradford,[4] Gironcoli,[5] and others. Gironcoli concludes that the results are chiefly: 1. Increase in the size of the kidney; 2. Generalized thickening of the capsule which he considers nontraumatic; 3. A dilatation of the arterioles and capillaries as seen in the microscopic section. Seres concludes that the changes are: 1. Increase in volume of the urine; 2. Decrease in the specific gravity due to a decrease in the solids per unit of volume, but 3. Little change in the total solids. 4. Earlier and more effective response to the diuretic effect of sodium chloride and glucose.

As a preliminary to a study of the effect of various agents upon the normal and denervated kidneys, we are reporting here the changes in the vascular bed as demonstrated by roentgenogram using the method described in a previous paper.

The denervation was accomplished as follows: Dogs were kept under Nembutal (Abbott) anesthesia, the kidney was exposed and delivered through an incision 2 cm. below and parallel to the costal margin and extending from a point about 3 cm. from the spinous

* The present investigation was aided by a grant from the Josiah Macey, Jr., Foundation.

[1] Seres, M., *Rev. Med. de Barc.*, 1924, **1**, 220. Abstracted *Z. f. Urol. Chir.*, 1925, **17**, 54.

[2] Ellinger, P. H., and Hirth, A., *Arch. f. Exp. Path. u. Pharm.*, 1925, **106**, 135.

[3] Bieter, R. N., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 792.

[4] Bradford, J. R., *J. Phys.*, 1889, **10**, 358.

[5] de Gironcoli, F., *Z. f. Urol. Chir.*, 1929, **27**, 26.

processes anteriorly for a distance of 5 to 8 cm. The peritoneal sheath over the kidney was divided and the pedicle fat and connective tissue stripped away. The macroscopic nerves were divided and the vessel sheaths removed. The renal artery was deprived of its adventia as far as this was possible by scraping between the blades of the hemostat.

After a period of 2 or more weeks the animals were utilized for injection of the renal vessels.

The injection, as was pointed out previously, filled the arterial

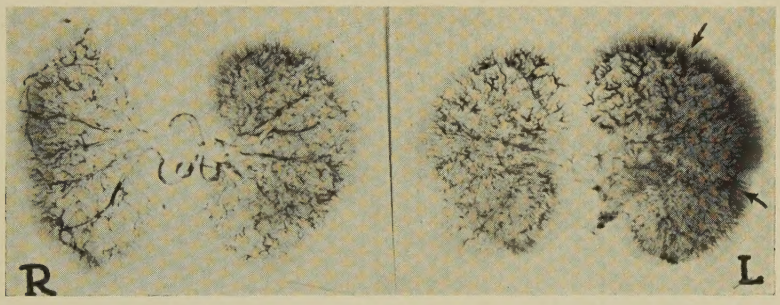

FIG. 1.

Injected kidneys, left kidney denervated two weeks prior to injection. Note the wider vascular bed of the left kidney, especially the increased number of arteriae recti.

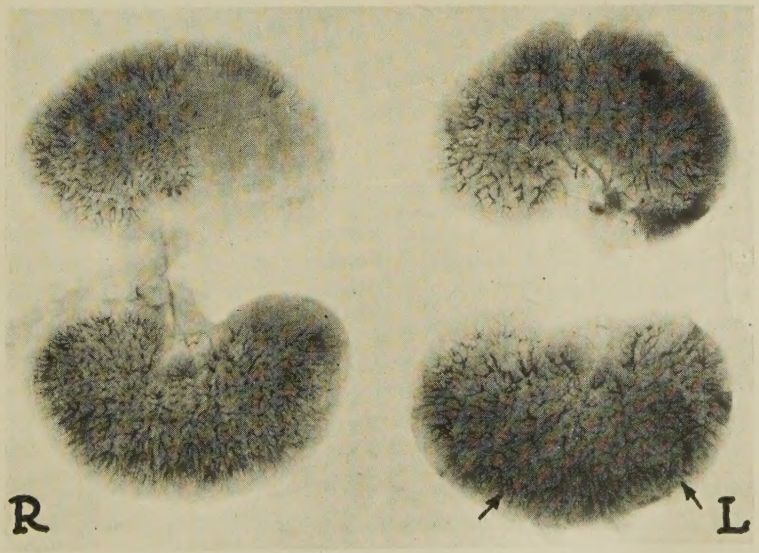

FIG. 2.

Injected kidneys, left kidney denervated three weeks prior to injection. Note the wider vascular bed of the left kidney, especially the dilatation of the arteriae recti, and lack of injection of one-half of one pole of the right kidney.

tree including the interlobular arteries with bismuth oxychloride to a sufficient degree to render them opaque to x-ray. The roentgenogram obtained was an accurate delineation of their outlines.

In interpreting changes the normal and denervated kidneys were compared as to the regularity in the outline of their vessels, the extent to which the interlobular vessels penetrated the cortex, and the relative number of vessels seen.

In general this series resulted in 2 types of pictures. The first, constituting the majority, showed the denervated kidney to be larger, the vessels to be more dilated, and the number and projection of the interlobular arteries to the outer margin of the cortex to be greater in the normal kidney. (Figs. 1, 2, and 3.) The sec-

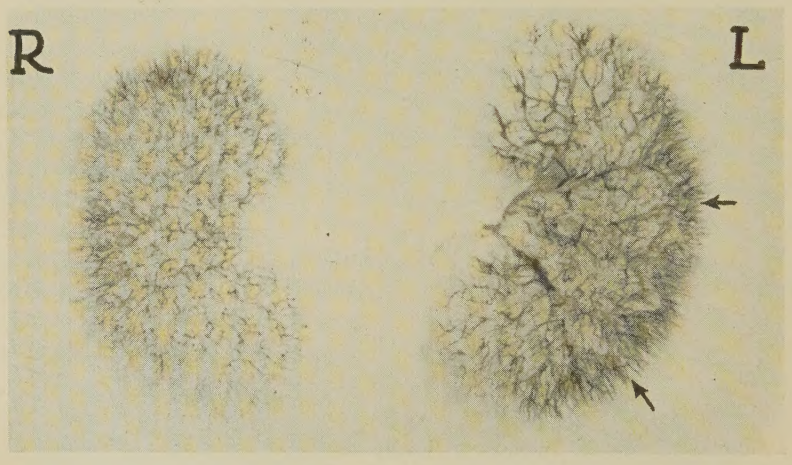

FIG. 3.
Injected kidneys, left kidney denervated forty-eight hours prior to injection. Note general vascular dilatation and particularly the larger arteriae recti and their more marked projection to the margin of the cortex.

ond group into which but few pictures fell was characterized by decrease in size and a marked decrease in the number of injected vessels in the denervated organ. The interlobular vessels extended only a short distance into the cortex in these specimens. In these, the denervated kidney was generally smaller than the normal. Correlation of the pictures with the histological findings showed in the first group dilatation of the interlobular vessels and glomerular tufts with the vessels and kidneys parenchyma well preserved. In the second group, vascular changes of a degenerative type such as endothelial swelling and rarely interstitial hemorrhages were found.

Conclusions. We have demonstrated in a pictorial manner the general types of vascular change resulting from denervation; (1) a

general dilatation of the vascular bed; (2) some degenerative changes affecting chiefly the blood vessel intima.

5311

Stability of Esterase and Ereptase in Ground Liver and Kidney Preserved in Glycerol.

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It was shown[1] that ground dog's liver preserved in pure glycerol had retained its peptone and ester-splitting powers unimpaired for a period of 34 months. In some instances it was found that the extract showed an even greater esterolytic power after standing in glycerol for nearly 3 years than after extraction for a period of 21 days.

The ground organs of these experimental animals have now been

TABLE I.

Animal	Organ	Esterolytic Activity After 21 days in Glycerol Shown in cc. 0.1 NaOH	Esterolytic Activity After 13 years in Glycerol Shown in cc. 0.1 NaOH	% Gain or Loss
Ph. 16d	Liver	3.27	3.53	+7.9
Ph. 16c	"	4.15	4.20	+1.2
Ph. 17c	"	3.70	3.55	—4
Ph. 16d	Kidney	2.25	1.36	—39.5
Ph. 16c	"	3.75	1.95	—48.0
Ph. 17c	"	2.65	1.38	—48.0
Ave.	Liver	3.71	3.76	+1.2
"	Kidney	2.88	1.56	—46.0

TABLE II.

Animal	Organ	Peptolytic Activity After 21 days in Glycerol Shown in cc. 0.1 NaOH	Peptolytic Activity After 13 years in Glycerol Shown in cc. 0.1 NaOH	% Gain or Loss
Ph. 16c	Liver	1.25	0.96	—23.2
Ph. 16c	"	1.45	1.05	—27.5
Ph. 17c	"	2.35	1.75	—25.5
Ph. 16d	Kidney	4.75	2.15	—54.5
Ph. 16c	"	4.20	2.28	—45.5
Ph. 17c	"	5.50	1.85	—70.0
Ave.	Liver	1.68	1.25	—25.5
"	Kidney	4.82	2.09	—57.0

[1] Simonds, J. P., *J. Exp. Med.*, 1918, **28**, 663; *Am. J. Physiol.*, 1919, **48**, 141.

standing in glycerol in a dark place, well corked for from 12½ to 13½ years. The esterolytic and peptolytic powers of the clear filtrates have again been tested using dilutions and technique similar to those of the original report.

The results of these experiments show that ground dog's liver preserved in glycerol retained its esterolytic activity unimpaired for 13 years, but lost about one-fourth of its peptone-splitting action during the same period. Ground dog's kidney similarly preserved for 13 years lost slightly less than half its original ester-splitting power and somewhat more than half of its former power to decompose peptone into amino acids.

5312

I. Influence of Eggwhite upon the Absorption of Bacteria from the Intestinal Tract.

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From the Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine, and Illinois State Department of Public Health, Chicago.

Friedberger[1] states that prolonged boiling of eggs, when fed exclusively, leads to severe trophic disturbances; Newburgh[2] fed eggwhite to rabbits and produced renal injuries after a short period of time. Galanini[3] fed only eggwhite to white rats and caused albumen in the urine before death; Friedberger and Abraham[4] state that egg diets frequently cause toxic effects. The harmful results of an exclusive egg diet are mainly due to the white of the egg (Stenquist,[5] Baglioni,[6] Bateman,[7] Iskikawa,[8] Arnold[9]). In this communication we report the results of experiments dealing with absorption through the blood stream.

The abdomens of 4 dogs, fasted for 24 hours, were opened under

[1] Friedberger, E., *Deut. Med. Woch.*, 1926, **52**, 1766.
[2] Newburgh, L. H., *Arch. Int. Med.*, 1919, **24**, 359.
[3] Galanini, Antonio, *Boll. Soc. Ital. Biol. Sperm.*, 1929, **4**, 91.
[4] Friedberger and Abraham, A., *Deut. Med. Woch.*, 1929, **55**, 383.
[5] Stenquist, F., *Deut. Med. Woch.*, 1928, **54**, 1920.
[6] Baglioni, S., *Boll. d. Soc. Ital di Biol. Sperm.*, 1928, **2**, 978.
[7] Bateman, G. W., *J. Biol. Chem.*, 1916, **26**, 263.
[8] Iskikawa, Issaka, *Jap. J. Med. Science*, 1928, **2**, 205.
[9] Arnold, L., *Am. J. Hyg.*, 1928, **3**, 604.

ether anesthesia and a suspension of *B. prodigiosus* (washings of 24 hours growth on agar plate in 50 cc. NaCl solution) was injected directly into the duodenum. Specimens of blood were taken from the portal vein, femoral vein and artery, every 5 minutes for half an hour, and plated on agar. After 24 hours of incubation at 37°C. the plates were read. In the specimens from the portal vein the results were always positive, and since we found the bacteria also in the femoral vein, though not in such large numbers, we took the specimens only from the femoral vein for subsequent experiments. Repeated puncture of the portal vein and the femoral artery caused hemorrhage and led to experimental errors.

Our experiments were conducted in the above mentioned manner. Suspension of bacteria was made up in NaCl and injected into the duodenum of 6 dogs; bacteria were suspended in one fresh raw eggwhite and injected into the duodenum of 8 dogs. Blood specimens were taken from the femoral vein at various intervals of time and the number of *B. prodigiosus* in the blood calculated per cc. (see chart). The dotted line represents the average amount of *B. prodigiosus* in the blood of dogs in which the micro-organisms were in NaCl solution, and the solid line—a raw eggwhite. The abscissa

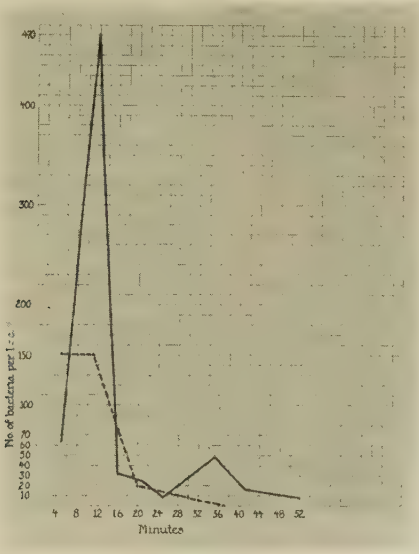

FIG. 1.

Ordinate = Number of bacteria per cc. of blood. Abscissa = Time in minutes.
- - - - - - - = *B. prodigiosus* in blood after saline suspension injected into the duodenum.
——— = *B. prodigiosus* in blood after eggwhite suspension injected into the duodenum.

shows time in minutes and the ordinate shows the number of bacteria per cc. of blood. The study of the action of eggwhite on the permeability of the intestinal wall is being continued.

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II. Influence of Eggwhite upon the Elimination of Bacteria into the Intestinal Tract.

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From the Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine, and Illinois State Department of Public Health, Chicago.

Raw eggwhite applied to the mucosa of the small intestine will influence the excretion of bacteria from the systemic circulation into the lumen of the small intestine. The preceding article dealt with the absorption of bacteria from the small intestine into the systemic circulation.

The dogs (25 animals) were fasted for 24 hours and operated upon under ether anesthesia. The abdomen was opened and different media were injected directly into the duodenum; normal salt solution used for 12 dogs, and fresh, raw eggwhite used, one eggwhite for each 13 dogs. The common bile duct was ligated and severed in order to exclude the passage of the bacteria into the duodenum by way of the bile. Ten cubic centimeters of a suspension of *B. prodigiosus* (one agar plate of *B. prodigiosus* suspended in 50 cc. of normal salt solution) were injected into the femoral vein and in 25 minutes the dogs were killed. Cultures were taken from the duodenum, upper and lower portions of the jejunum, ileum and caecum, with a sterile swab and smeared on agar plates. These were incubated at 37° for 24 hours. The results showed that *B. prodigiosus* passed through the wall of the intestine and appeared in greater numbers in the duodenum and upper portion of the jejunum, while a far less number was noted in the lower portion of the jejunum and practically none in the ileum and caecum.

The greatest number of bacteria appeared in the dogs in which eggwhite had been introduced into the duodenum, while a much less number appeared in the dogs injected with the saline suspension.

The accompanying chart shows the results of the experiments. The abscissa represents the segments of the intestinal tract and the ordinate the number of colonies grown on the agar plates inoculated

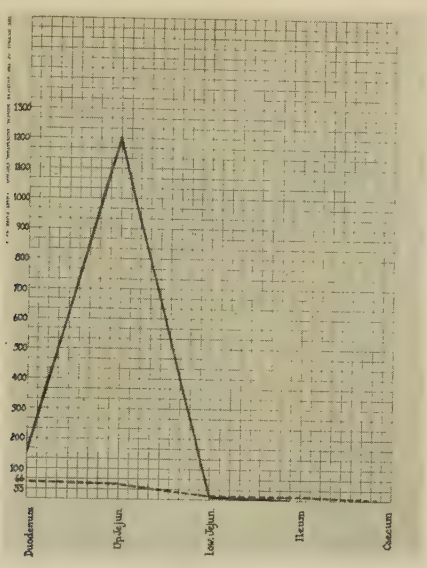

FIG. 1.

Ordinate = Number of viable bacteria. Abscissa = Segments of intestinal tract.
- - - - - - - = Saline suspension injected into duodenum.
——————— = Eggwhite suspension injected into duodenum.

with swabs. The solid line shows the results of the dogs which had been injected intraduodenally with eggwhite, and the dotted line of those injected intraduodenally with the saline.

The study of the action of eggwhite on the permeability of the intestinal wall is being continued.

5314

III. Influence of Eggwhite upon the Cyclic Circulation of Bacteria in the Splanchnic Area.

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Having shown in the 2 preceding articles that eggwhite increases the permeability of the intestinal wall for living bacteria from the intestinal tract into the circulation as well as from the blood stream into the intestinal tract, the question arose whether it is not possible that the bacteria might be absorbed and eliminated at the same time;

that is, can living bacteria be absorbed from one segment and eliminated into another part of the intestine by means of the circulation? Also, what effect would eggwhite have to do with this cyclic circulation of bacteria?

Thirty dogs, fasted for 24 hours, operated upon under ether anesthesia, were used for these experiments. The technic consisted of opening the abdomen, ligating and severing the lower part of the duodenum. Twenty-five cubic centimeters of a suspension of *B. prodigiosus* or *B. murii* were injected into the duodenum in the first series, and 50 cc. into the upper part of the jejunum in the second series. The suspension of bacteria was made up in a normal salt solution in one series and in one fresh raw eggwhite in the other series. The same media without the bacteria were simultaneously injected into the end adjacent to the ligation to produce the same state in the mucous membrane of the segment in which the bacteria were injected. The dogs were killed in 25 minutes, the various segments of the gut were examined for the presence of the test bacteria with sterile swabs which were smeared on agar plates.

Twice as many dogs were used for this experiment as we are reporting. All experiments in which blood was detected in the ligated segments examined were discarded.

The first series in this group of experiments (18 dogs) con-

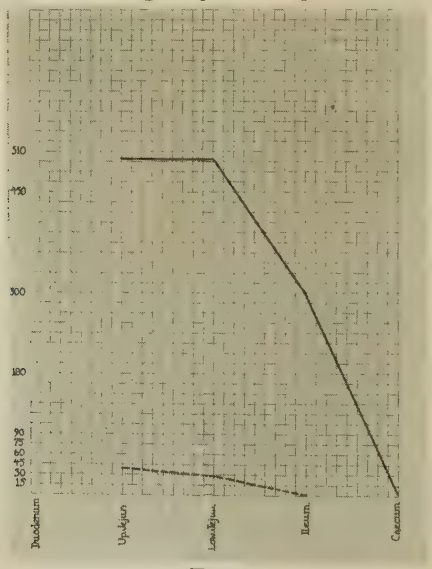

FIG. 1.

Ordinate = Number of bacteria on agar plates.
Abscissa = Segments of the intestinal tract.
- - - - - - - = Saline suspension injected into duodenum.
——————— = Eggwhite suspension injected into duodenum.

sisted in injecting bacteria into the duodenum. The duodenum, upper jejunum, lower jejunum, ileum and caecum were examined for the presence of the test bacteria. Fig. 1 gives the results of the experiments. In the duodenum there was always a full growth of bacteria, so it was not recorded on the figure. The abscissa represents the segments of the intestinal tract and the ordinate the number of colonies on agar plates. The solid line shows the results in the dogs where the eggwhite was injected; and the dotted line where the saline suspension was used.

The second series (12 dogs) consisted in injecting the test bacteria into the upper jejunum, and examining the duodenum for the presence of these bacteria. In this case the common bile duct was also ligated and severed to exclude the possibility of bacteria descending from the liver. The accompanying Fig. 2 shows the re-

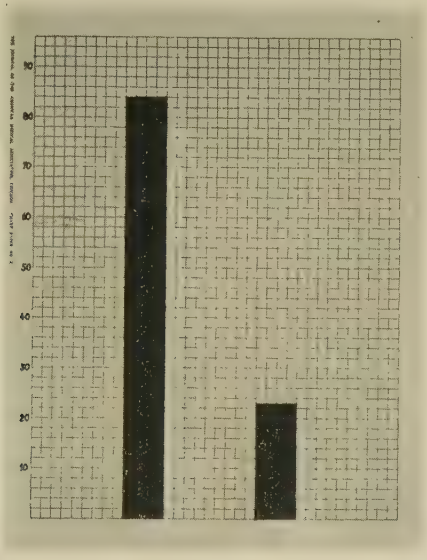

FIG. 2.
Ordinate = Average number of bacteria appearing in the duodenum.
Tall Column = Eggwhite suspension injected into jejunum.
Short Column = Saline suspension injected into jejunum.

sults of the experiments. The higher column shows the relative amount of bacteria (see ordinate) in the duodenum of the dogs where the eggwhite was used, and the shorter one, the saline suspension. The plates from the upper jejunum showed, of course, a full growth and were not recorded.

These experiments tend to show that in the dog bacteria can be absorbed from the duodenum and excreted into the jejunum. Bac-

teria can also be absorbed from the jejunum and excreted into the duodenum. Raw eggwhite increases the number of bacteria excreted in each series of experiments.

The study of the effect of eggwhite on the permeability of the intestinal wall is being continued.

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IV. Influence of Eggwhite in the Duodenum upon the Elimination of Bacteria into the Gallbladder.

A. J. NEDZEL AND LLOYD ARNOLD.

From the Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine, and Illinois State Department of Public Health, Chicago.

Further study of the action of a fresh raw eggwhite upon the cyclic circulation of bacteria in the splanchnic area, led us to examine the gallbladder for bacteria after intraduodenal injection. The following experiments were carried out:

The abdomens of 27 dogs, fasted for 24 hours, were opened under ether anesthesia and the cystic duct ligated and severed. Twenty-five cubic centimeters of a suspension of *B. prodigiosus* in saline solution (washings of 24 hours' growth on agar plate in 50 cc. of saline solution) was injected directly into the duodenum in 15 dogs. In the other 12 dogs the *B. prodigiosus* was injected with a fresh raw eggwhite, one for each dog. In 30 minutes the dogs were killed and the bile from the gallbladder was poured directly into a large flask of broth. The results were recorded 24 hours after incubating the cultures at 37°C. The results are shown in an accompanying chart where the ordinate shows percent of positive results (appearance of *B. prodigiosus* in gallbladder), the higher column representing the experiments where the eggwhite was used and the lower, the saline solution.

The technic used in these experiments may be criticized, since the hemato-hepatogenous route of the infection of the gallbladder is generally accepted (Meyer[1]). But one cannot also overlook the statements of some investigators, who also accept the probabilities of infection of the gallbladder through the lymph and blood vessels (Gay,[2] Chirolanza[3]). The opponents though (Meyer[1]) of the

[1] Meyer, K. F., Neilson, N., and Fensier, *J. Infect. Dis.*, 1921, **28**, 456.

[2] Gay, F., Typhoid Fever, 1918.

[3] Chirolanza, R., *Z. f. Hyg.*, 1909, **62**, 11.

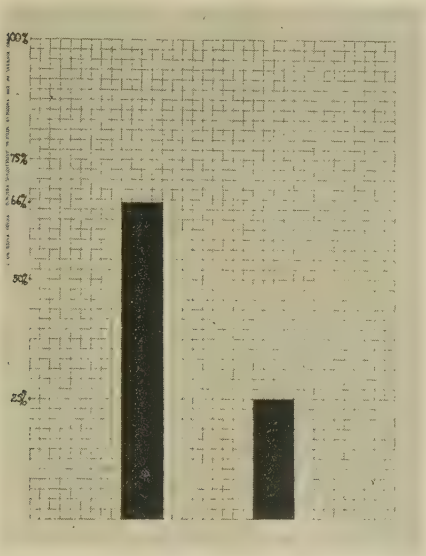

FIG. 1.

Ordinate = Percent of experiments in which *B. prodigiosus* appeared in gall bladder bile.
Tall Column = Eggwhite suspension injected into duodenum.
Short Column = Saline suspension injected into duodenum.

latter opinion state that "technically the experiments have been poorly executed." This technic consisted in tying off the cystic duct of rabbits and injecting typhoid bacilli intravenously, immediately following the operation. Meyer states, "in the ligating of the cystic duct the accompanying cystic artery was in all probability also tied and hemorrhagic infarction occurred from incomplete collateral circulation." Doer[4] also tied off the cystic duct but waited several days before injecting the bacilli intravenously. This result was negative, but we must take into consideration that he used only one rabbit. Johannes[5] states that experimentally it has been established that *Staphylococcus aureus* is eliminated into the gallbladder via the hematogenous route. Koch[6] proves histologically that the bacilli get into the gallbladder through the wall by the blood vessels. Fraenkel[7] says that *B. typhosus* gets into the gallbladder through its vessels and through the liver, but cannot decide which route is the main one.

So we repeated Doer's experiments on 4 dogs with extreme pre-

[4] Doer, R., *Centrollblatt für Bacteriologie*, 1905, **39**, 624.
[5] Johannes, F., *Arch. f. Klin. Chirurg.*, 1927, **144**, 369.
[6] Koch, J., *Ztrsch. f. Hyg.*, 1909, **62**, 1.
[7] Fraenkel, E., *Mitt. aus den Grenzgebieten der Mediz. u. Chir.*, 1909, **20**, 898.

cautions. These dogs were operated upon under ether anesthesia and under sterile conditions. After opening the abdomen the cystic duct was tied off close to the common bile duct, carefully avoiding injury of gallbladder vessels. Two of these dogs were killed on the seventh day, the third dog on the eighteenth day and the fourth dog on the twentieth day. Half an hour before killing these animals 5 cc. of a saline suspension of *B. prodigiosus* (washings of one agar plate in 50 cc. of a saline solution) were injected into the femoral vein of each dog. The bile, *in toto,* was removed under sterile precautions and placed in a flask of broth. The swabs, after swabbing the wall of the gallbladder, were also used to inoculate the broth. All 4 dogs gave positive results, showing the presence of *B. prodigiosus.* We conclude that the hematogenous route also plays a part in the elimination of bacteria into the gallbladder.

5316

V. Absorption of Bacteria from the Gallbladder.

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From the Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine, and Illinois State Department of Public Health, Chicago.

In the preceding reports we have observed a cyclic circulation of bacteria in the splanchnic area of the body. The gallbladder was involved in this cycle so we next studied the absorption of bacteria from the gallbladder into the splanchnic circulation.

It has long been known that resorption of neutral fats, lipoids, bile pigment and other substances through the epithelium and lymphatics of the gallbladder mucosa can be demonstrated histologically (Royster[1]). In long continued closure of the cystic duct the cholesterol constituent is reduced from resorption by the bladder wall (Rosenthal and Licht[2]). Blad[3] and Bundschuk[4] described cases of bile peritonitis where, according to their investigations, there was no perforation of the gallbladder. Lange and Roos[5] performed experiments in which, after injection of bacilli into the gallbladder,

[1] Royster, H. A., *Med. J. and Rec.*, 1930, **132**, 232.

[2] Rosenthal and Licht, *Klin. Woch.*, 1928, **7**, 1952.

[3] Blad, A., *Arch. f. Klin. Chir.*, 1917, **109**, 101.

[4] Bundschuk, E., *Arch. f. Klin. Chir.*, 1930, **161**, 549.

[5] Lange and Roos, *Arch. Kais. Gesdhamt*, 1917, **50**, 57.

the same bacteria was demonstrated in the ear vein of a rabbit as soon as one or 2 minutes after injection.

Our experiments were as follows: The abdomen of 6 dogs, fasted for 24 hours, had been opened under ether anesthesia. A cut in the common bile duct was made large enough to insert a No. 6 silk catheter. The latter was introduced through this opening, through the cystic duct into the lumen of the gallbladder. Then the cystic duct was ligated close to the common bile duct, in order not to injure the cystic artery and vein and at the same time keep the catheter in place. (See the schematic drawing.)

FIG. 1.

The bile was drawn out with a syringe through the catheter and replaced with 5 cc. of a suspension of *B. prodigiosus* in saline solution (washings of agar plate in 25 cc. of *B. prodigiosus*). The dogs were killed in 30 minutes and 3 pieces of liver from different parts were cut out and placed into 100 cc. flask of broth. Sterile gauze was packed around the area of the incision in the common bile duct through which the catheter was inserted. This gauze was removed after the animal was sacrificed and in all 6 experiments *B. prodigiosus* could not be cultivated from the gauze. In 4 dogs the results were positive for the presence of *B. prodigiosus* in the liver and in 2, negative. These experiments show that there is absorption of bacteria from the gallbladder into the splanchnic circulation in the majority of the animals.

5317

Effect of Rectally Administered Ether-Oil Mixtures on Absorption of Histamine from the Colon.

R. W. ALBI AND T. E. BOYD.

From the Department of Physiology and Pharmacology, Loyola University School of Medicine.

Koessler and Hanke[1] showed that histamine and other amines are produced by bacterial activity in the intestine, and that histamine is apparently detoxified during absorption through the intestinal wall. Best and McHenry[2] have found in the intestinal mucosa of the dog an enzyme which destroys the physiological activity of histamine. It seems, therefore, that the process of detoxication may be an important function of the normal mucosa.

In an earlier paper from this laboratory[3] it was shown that exposure to certain chemical agents renders the dog's intestine permeable to histamine. Such an effect was noted with alcohol and chloroform, in varying dilutions. It therefore seemed of interest to study the effect of ether, because of the extensive use of ether-oil mixtures given per rectum for anesthesia.

Small dogs, in good health, were used. Preparation included 18 hours without food, 10 mg. per kilo of morphine sulphate given subcutaneously, and an enema of warm tap water. Anesthesia was induced by ether inhalation, which was lightened subsequently whenever additional ether was given per rectum. Records were made of respiration and of carotid blood pressure. A soft rubber catheter and draining tube were fixed in place for rectal injections. The dose of ether-olive oil mixture was 3 cc. per kilo body weight, as in the experiments of Beckmann, cited by Gwathmey.[4] The ratio by volume of ether to oil was varied from 75/25 to 35/65. After retention from 5 to 20 minutes the residue was washed out with saline solution. Five to 10 minutes later histamine dichloride (Eastman), 5 mg. per kilo in saline solution, was allowed to run into the colon.

Under these conditions, the administration of histamine was followed in every instance by an immediate and marked fall of arterial pressure. The general curve was similar to those shown in

[1] Koessler, K. K., and Hanke, M. T., *J. Biol. Chem.*, 1924, **59**, 889.

[2] Best, C. H., and McHenry, E. W., *Am. J. Physiol.*, 1930, **93**, 633.

[3] Mammoser, L. F., and Boyd, T. E., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 765.

[4] Gwathmey, J. T., *J. Am. Med. Assn.*, 1929, **93**, 447.

the paper of Mammoser and Boyd.[3] The degree and duration of the depressor effect varied with the concentration of ether used. The maximum fall observed was from 128 to 76 mm., after retention of 75% ether for 5 minutes. In this instance the pressure after 48 minutes was still 12 mm. below the original level. The smallest fall recorded was from 126 to 112 mm., after retention of 35% ether for 20 minutes. The pressure remained below the original level for only 14 minutes.

In control experiments an enema of olive oil alone, or of saline, was substituted for the ether-oil mixture. Histamine subsequently administered did not affect the arterial pressure.

In all the concentrations used, therefore, ether appears to render the epithelium of the large bowel permeable, in some degree, to histamine. The normal impermeability, however, is largely or completely regained within 24 hours. Two dogs received the routine morphine injection and warm-water enema. A 65/35 ether-oil mixture was given by catheter and retained for one hour, the residue being washed out with saline. The dogs were allowed to recover, and 24 hours later were anesthetized by ether inhalation. The blood pressure was recorded and the usual dose of histamine given by catheter. In one dog the pressure was entirely unaffected, in the second there was a transitory fall of only 8 mm.

Some hyperemia was noted in the colon mucosa of 7 out of 8 dogs receiving ether and oil plus histamine. The mucosa in the control animals appeared normal, so the hyperemia was not due to histamine alone.

The amounts of histamine which we used were presumably larger than would ever exist under normal conditions at any one time in the bowel. In view of the marked depressor action, however, it seems probable that much smaller amounts would have produced effects of physiological importance. There is also a possibility that ether and similar agents may affect the absorption, or detoxication, of other potentially toxic substances besides histamine.

5318

Hydrolysate of Proteins as the Basis for a Bacteriological Culture Medium.*

ALDEN K. BOOR AND C. PHILLIP MILLER, JR.

From the Department of Medicine, University of Chicago.

The advantage of a medium, free from antigenic proteins and suitable for the growth of organisms, particularly pathogenic bacteria, is obvious.

Long and Seibert[1] introduced a synthetic, non-protein medium suitable for growth of tubercle bacilli. ZoBell and Meyer[2] have described the adaptation of the Brucella group to a protein or peptone-free environment. Miller and Castles[3] have found that a tryptic digest of egg white, from which the coagulable proteins have been removed, is an excellent basis for a medium for gonococcus.

Twenty grams of commercial, dried, powdered egg white were subjected to the action of 50 cc. of boiling, 20% hydrochloric acid for 10 hours, using a reflux condenser for the purpose. It was found that other proteins, *e. g.*, casein and gelatin, could be substituted for the egg white. Excess hydrochloric acid was removed from the hydrolysate by evaporation to a thick paste and the product diluted with water to 500 cc. This solution was treated with 63 cc. of a solution containing 10.35% sodium hydroxide, 0.67% potassium hydroxide; and 0.03% calcium hydroxide. This composition was chosen to insure the presence of the salts of these metals in a quantity to best meet bacterial requirements. The solution was filtered. About one-fifth of its volume of a carefully prepared aluminum hydroxide cream† was added to precipitate some colloidal matter, and the solution again filtered. The volume of this clear, transparent filtrate was then brought to 1,750 cc. with distilled water and 0.10 gm. Na_2HPO_4 and 0.02 gm. NaH_2PO_4 added for buffer effect. One percent dextrose was added and in the case of the solid medium, 2% agar. The final adjustment of the reaction to pH 7.4 was made by addition of the hydroxide mixture.

* This study was made possible by a research grant from the Public Health Institute of Chicago.

[1] Long and Seibert, *Am. Rev. Tuber.*, 1926, **13**, 393.

[2] ZoBell and Meyer, *Science*, 1930, **72**, 176.

[3] Miller and Castles, PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 123.

† The aluminum hydroxide cream was prepared by adding slowly and with constant stirring a 1% solution of ammonium hydroxide to a 1% solution of ammonium alum and repeatedly washing the precipitate by decantation until the supernatant liquid no longer gave a reaction with Nessler Reagent.

That the medium was free from those proteins generally agreed to be antigenic is indicated by the following negative tests: biuret reaction (after removal of ammonium salts), sulfosalicylic acid, picric acid and trichloracetic acid. No precipitate appeared when the solution was half saturated or completely saturated with ammonium sulfate.

The following organisms have multiplied on the medium herein described; gonococcus, *Staphylococcus aureus, B. coli, B. paratyphosus A, B. paratyphosus B, B. fecalis alkaligines,* pneumococcus, *B. typhosus, B. abortus, B. melitensis, B. dysenteriae, B. anthracis, B. diphtheria,* meningococcus, and others. Some of these organisms have exhibited a luxuriant growth. Others have multiplied to a lesser extent.

5319

Effect of Various Stomach Preparations in Pernicious Anemia.

J. P. BURGESS AND J. E. MORGAN. (Introduced by A. C. Ivy.)

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

This work was started in April, 1929, under the direction of Dr. Ivy on the principle that the active constituent of liver effective in pernicious anemia might be produced by the gastric mucosa and stored in the liver. Very soon after starting our work articles appeared showing that desiccated whole stomach is effective,[1, 2] that gastric mucosa is slightly effective, whereas gastric muscle is ineffective,[1] that a normal *in vivo* digest of meat is effective,[3] that pepsin is ineffective,[3] and that gastric juice is ineffective.[4, 5] Our report is simply to record a confirmation of some of these findings.

We have fed to pernicious anemia patients fresh hog's gastric mucosa (300 gm. daily) brought to a boil within 20 minutes, pepsin (75 gm. of scale pepsin daily), desiccated mucosa (prepared by the method of Sturgis, Isaacs and Sharp, 75 gm. daily or the equivalent of 450 gm. of fresh mucosa), desiccated gastric muscle (120 gm. daily or the equivalent of 450 gm.) and desiccated whole stom-

[1] Sturgis and Isaacs, *J. Am. Med. Assn.*, 1929, **93**, 747.
[2] Sharp, *J. Am. Med. Assn.*, 1929, **93**, 10.
[3] Castle, *Brit. Med. J.*, 1929, **1**, 1120.
[4] Castle and Townsend, *Am. J. Med. Sci.*, 1929, **178**, 748.
[5] Coggeshall, PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 1044.

ach (120 gm. daily or the equivalent of 450 gm.). It should be pointed out that the preparation of our material (except pepsin) was started within one hour after death of the hog, and that during the process of desiccation there is a considerable opportunity for autolysis to occur which was not the case in the preparation of the fresh hog's mucosa in which the enzymes were destroyed with heat.

Three patients did not respond to the fresh hog's mucosa, but did to liver. Two patients did not respond to pepsin, but did to liver. Of 4 patients on desiccated mucosa, one responded definitely but slowly; 3 did not respond, but stated that they felt better, and later responded to liver. One patient on desiccated gastric muscle remained stationary for 2 months. Three patients responded definitely and typically to desiccated whole stomach. (By response we mean a definite increase in reticulocytes and red cells within 2 weeks after the institution of therapy.)

Our observations confirm those of Sturgis and Isaacs, namely, that a small amount of the anti-pernicious anemia principle is present in gastric mucosa, very little, if any, is present in gastric muscle, and that when the whole stomach is ground and desiccated, a considerable quantity of the active principle is produced or liberated, most probably by autolysis.

5320

Influence of Gastric Acid Secretion upon the Bactericidal Power of the Gastro-Intestinal Tract.

S. F. FURBY AND LLOYD ARNOLD.

From the Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine, and Research Laboratory of State Department of Public Health, Chicago.

The bactericidal power of the free H-ion of the gastric contents has been thought to be the principal disinfecting agent. We have used *B. prodigiosus* as the test bacteria. Suspensions of one agar plate growth in 100 cc. saline were administered to dogs by stomach tube. Alcohol was used to stimulate gastric secretion. Fifty cc. of a 7% ethyl alcohol were administered by stomach tube. Ninety-six dogs were used for these experiments. The number were equally divided in each experiment as nearly as possible. All animals were without food for 24 hours before the experiments began. The accompanying table shows the results. All animals were killed 2

hours after the administration of the material. The lumen of the stomach and small intestine was examined immediately by transferring as uniform an inoculum from the contents of the various levels on to the surface of agar plates. Bent glass spreaders were used for uniform distribution and the same spreader was used to smear other sterile agar plates for dilutions in case of overgrowth. Where the *B. prodigiosus* appeared in the same concentration as was present in the original suspension, the growth was recorded as 100%.

TABLE I.

Effects of Gastric Acidity and Manipulation of the Duodenum upon the Self-Disinfecting Power of the Stomach and Small Intestine.

B. prodigiosus in saline	Gastric Acidity	Stomach	Duodenum	Upper Jejunum	Lower Jejunum	Ileum
Without alcohol	Free					
	Deficit	20%	20%	40%	70%	90%
Plus alcohol at	Free	0	0	0	0	0
same time	Deficit	10%	10%	40%	20%	0
Plus alcohol	Free	0	0	0	0	0
30 minutes after	Deficit	30%	50%	50%	60%	0
Plus alcohol	Free	0	0	0	0	0
30 minutes before	Deficit	10%	40%	30%	20%	0
Alcohol in stomach						
and *B. prodigiosus*	Free	10%	40%	20%	10%	0
in duodenum	Deficit	20%	20%	20%	0	0
Alcohol and *B. prodigiosus* in stomach						
and sterile saline in	Free	20%	20%	30%	20%	0
duodenum	Deficit	40%	20%	20%	0	0

The dogs showing a free acidity and a deficit of free acid after the 2 hour period are recorded separately in the table. One could conclude that where free acid was present, the administered bacteria were not viable within the stomach or small intestine after 2 hours.

The last 2 experimental results shown on the table do not substantiate the above statement. The alcohol test meal was given by stomach tube. The dogs were given general anesthetic, duodenum opened, *B. prodigiosus* in saline was injected directly into the duodenum. Two hours later the animal was killed. There does not seem to be any relationship between gastric acidity and survival of ingested bacteria. The last part of the table records the results obtained after administering the bacterial suspension and the alcohol test meal in the usual way, both at the same time. The abdomen was opened under general anesthesia and sterile saline injected into the lumen of the duodenum. As in the previous experiment, these results show no apparent relationship between gastric acidity and

the survival of ingested bacteria. The manual manipulation of the intestinal tract seems to be an important factor in these experiments in inhibiting the bactericidal power. Free acid as high as 60 was observed in some of the last recorded experiments with viable *B. prodigiosus* after 2 hours.

5321

Influence of Broth Cultures and Media upon the Self-Disinfection of the Skin.

B. E. MONTGOMERY. (Introduced by Lloyd Arnold.)

From the Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine, and State Department of Public Health, Chicago.

Arnold, Gustafson, Hull, Montgomery and Singer[1] reported upon the disappearance of viable bacteria from the skin when applied in 1:200 saline dilution. This suspension was chosen after varying concentrations had been applied to the skin. Pease, and Himebaugh[2] reported some observations using undiluted broth cultures of bacteria under certain conditions. These workers overloaded the skin with the foreign solids in the broth. The air drying of the skin causes a concentration of the protein and other materials in the broth upon the skin and delays its self-disinfecting power. The undiluted 24-hour-old broth cultures contain more bacteria than the skin can remove in 15 minutes, but the foreign substances covering the cornified layer are more important in the reaction than the concentrations of the bacteria.

The middle finger of both hands was submerged in the various fluid media indicated in the table. Immediately after removal the

TABLE I.

24-hour Broth Culture	% of Viable Bacteria Destroyed.
Undiluted	25
Diluted 1:10 (saline)	35
" 1:200 "	90
Sterile broth, air dried, submerged in 1:200 (saline)	32

Fingers submerged in suspensions of *B. prodigiosus*. Dried in air for 15 minutes and palmar surface pressed against agar plate.

[1] Arnold, L., Gustafson, C., Hull, T. G., Montgomery, B. E., and Singer, C., *Am. J. Hyg.*, 1930, **11**, 345.

[2] Pease, H. D., and Himebaugh, L. C., *Am. J. Pub. Health*, 1930, **20**, 820.

palmar surface of one finger was pressed against the surface of a sterile agar plate and smeared with a bent glass spreader. After holding the finger of the other hand free and allowing it to dry for 15 minutes, the same procedure was followed. The first culture was taken as the initial contact dose. Healthy subjects must be used for these experiments. If female subjects are used, care must be taken to avoid certain periods during the menstrual cycle, inasmuch as the disinfecting power of the skin can vary 10 to 15% at times. The skin of diabetic patients has approximately half of the disinfecting power of normal skin.

Care must be exercised in testing the physiological variations in self-disinfecting power of the skin to avoid placing a layer of foreign material over the cornified epithelium and prevent contact of bacteria with this layer. The results reported here substantiate earlier publications from this laboratory.

5322

Optimum Bacterial Suspension for Testing Skin Disinfection.

R. KARNS AND LLOYD ARNOLD.

From the Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine, and State Department of Public Health, Chicago.

Singer and Arnold[1] and Arnold, Gustafson, Hull, Montgomery and Singer[2] used 1:200 dilutions of broth cultures of bacteria in saline for testing the self-disinfecting power of the skin. We have found that this is not the optimum concentration to be used for this purpose. The 1:200 suspension is too dilute to test this function. We have found that it is necessary to increase the concentration of bacteria in the suspension and to extend the period of the test over 60 minutes instead of 30 minutes. The 1:200 suspension is so dilute that there can be a considerable variation in the relative self-disinfecting power and still show a 100% destruction of the test bacteria. The accompanying table gives the results of 250 experiments, 50 experiments for each dilution are averaged. The variations for each dilution are on the average of less than 5%. The technic was the same as that used by Arnold, *et al.*[2]

[1] Singer, C., and Arnold, L., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 364.

[2] Arnold, L., Gustafson, C., Montgomery, B. E., Hull, T. G., and Singer, C., *Am. J. Hyg.*, 1930, **11**, 345.

TABLE I.
Percent Destruction of Viable B. prodigiosus from Palmar Surface of Hand.

24-hour Broth Culture Diluted with Saline	After				
	Immediate	15 min.	30 min.	45 min.	60 min.
1:10	0	40	60	65	70
1:50	0	60	75	86	91
1:100	0	68	86	98	99
1:200	0	74	98	99	100
1:500	0	90	99	100	100

We have found that the 1:50 dilution is the best for testing the self-disinfecting power of the skin and the time extended over one hour. The reserve self-disinfecting power can be determined if this concentration is used. Palmar and dorsal surfaces of the hand show constant differences, the female's skin varies during certain periods of the menstrual cycle, pathological skin shows considerable variations by this method. These results will be reported in full in the near future.

5323

Effect of Bilateral Suprarenalectomy on Certain Constituents of the Blood of Dogs.

A. B. HASTINGS AND E. L. COMPERE.

From the Lasker Foundation for Medical Research and the Department of Medicine and the Douglas Smith Foundation for Medical Research and the Department of Surgery, University of Chicago.

A study of the changes in the blood of dogs following bilateral suprarenalectomy has been made to attempt to determine what important constituents undergo pathological changes in their concentration. Data have been previously available suggesting pathological changes, but many of the results have been conflicting and no day by day analyses have been made. Accordingly, analyses have been made before operation, after removal of one suprarenal gland, and daily observations after removal of the second gland until death occurred, from 4 to 14 days later. The constituents which have been studied on 15 dogs are: the pH and the CO_2 content of the blood serum, in order to determine whether or not there was a pathological change in the acid-base balance; sugar and lactic acid concentration of the blood serum, in order to determine whether a

change in the carbohydrate metabolism had occurred; calcium and potassium concentration of the serum, in order to determine whether these important, related, inorganic cations had changed in their concentration; the serum creatine and creatinine, because of the suspected relation between muscle activity and suprarenal insufficiency; the serum proteins and percentage of erythrocytes in the blood, in order to determine whether concentration of the blood or blood serum occurred.

Briefly, the results of this investigation may be summarized as follows: The *bicarbonate of the serum* fell progressively from a normal value of 20 mM. per liter to the low value of 9 mM. per liter at death. *pH* falls but slightly prior to the terminal stages and then becomes as low as 7.1. The *blood sugar* is usually slightly lower immediately following the removal of the second suprarenal although it has sometimes been found equally low following the removal of one suprarenal. The drop is not marked, however, the serum concentration being approximately 90 mg. per 100 cc. as compared with the normal serum value of 120 mg. per 100 cc. Contrary to other investigators' reports, there is no further drop in the serum sugar following the removal of the second suprarenal gland. In contradistinction to the lack of changes in the serum sugar, however, there is a marked and consistent drop in the serum *lactic acid* the day following the removal of the second suprarenal gland. It reaches a value of half its normal concentration and remains low until the terminal stages set in. The *serum calcium* remains practically normal rising slightly as the blood serum becomes more concentrated. It is felt that this slight increase in calcium concentration can be wholly accounted for by the increase in concentration of the serum proteins and has no direct relation to the removal of the suprarenal glands. The *serum potassium,* however, increased markedly within 48 hours after the removal of the second suprarenal gland. The rise may be as much as 50% on the second day. The rise continues until the very high value of approximately 20 mM. per liter is reached at death compared with a normal value of 3 mM. per liter. It is believed that this observation of the very high terminal concentration of potassium which has also been found to be approximately the concentration of potassium which results in the death of normal dogs when potassium chloride is injected intravenously, may be of importance in reaching an understanding of the cause of death following the functional or surgical removal of the suprarenal glands. The creatine and creatinine showed no changes until 24 hours before death when there occurred a terminal

rise due presumably to an impairment of the kidney function and the retention of urine. The percentage of red cells in the blood remained remarkably constant throughout the period from the removal of the second suprarenal gland until death. There was, however, a progressive increase in the concentration of the serum proteins reaching a maximum concentration approximately 25% higher than the normal concentration.

5324

I. Effect of Suprarenalectomy on Muscle Tissue Respiration.

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From the Lasker Foundation for Medical Research and the Department of Medicine, University of Chicago.

A series of 25 experiments were performed on as many suprarenalectomized, male mice and their normal, male litter mates in order to determine the effect of suprarenalectomy on the respiration of excised skeletal muscle. The tissue respiration was measured on

TABLE I.

Showing Comparison of the Respiration of Excised Abdominal Muscle of 13 *Pairs of Normal and Suprarenalectomized Mice.*

No. of Exp.	Days after operation	Cu. mm. O_2 consumed per mg. per hr.		Cu. mm. of Extra CO_2 Produced Aerobically per mg. per hr.		Cu. mm. of CO_2 Produced Anaerobically per mg. per hr.	
		Normal	Operated	Normal	Operated	Normal	Operated
1	55	4.78	5.80	2.54	2.88	5.74	7.54
2	56	4.63	6.39	2.63	3.85	6.89	9.12
3	59	5.46	7.17	2.77	3.16	4.71	6.50
4	61	3.72	7.06	2.33	3.04	3.35	8.80
5	63	4.92	6.70	2.58	3.45	4.36	7.60
6	65	4.83	6.38	3.49	3.39	3.99	5.75
7	66	3.85	6.13	1.85	2.55	3.87	6.90
8	67	4.58	5.58	2.63	2.88	6.48	7.22
9	67	4.13	5.81	1.91	2.83	4.03	6.50
10	67	6.12	6.84	3.46	3.51	4.30	6.16
11	68	3.98	5.03	3.26	3.33	3.57	5.46
12	68	5.01	7.45	2.70	3.53	3.75	8.10
13	69	4.75	7.23	2.57	3.94	4.57	7.10
Means		4.67	6.43	2.67	3.26	4.59	7.14
Difference of means		1.76		0.59		2.55	
Probable error of difference of means		0.19		0.13		0.28	

abdominal muscle by a modification of the Warburg differential method.

The accompanying table shows the values obtained in 13 of these experiments. The quantity of oxygen consumed is expressed in terms of cubic millimeters consumed during the first hour of respiration per milligram of dried tissue. Columns 5 and 6 compare the aerobic production of lactic acid by muscle of normal mice and of suprarenalectomized mice. Columns 7 and 8 compare the anaerobic production of lactic acid by muscle of normal mice and of suprarenalectomized mice. The quantity of lactic acid produced both aerobically and anaerobically is expressed in accordance with the Warburg method in terms of cubic millimeters of carbon dioxide produced during the first hour of respiration per milligram of dried tissue. At the foot of the table is given first, the means of these 6 groups of values; second, the difference of the means for the groups compared; third, the probable error of the difference of the means.

The 13 experiments show respiration values consistently greater for the suprarenalectomized mice. This difference applies only to the period starting about the 55th day and ending about the 70th day after suprarenalectomy. Before and after that period there is no difference in the respiration of the muscle of the normal and suprarenalectomized mice.

5325

Administration of Viosterol in Human Parathyroid Tetany.

LINDON SEEDS AND C. I. REED.*

From the Departments of Surgery and Physiology, University of Illinois College of Medicine.

It has been shown by several investigators that preoperative administration of viosterol or irradiation, which presumably accomplishes the same ultimate result, will protect dogs to some extent against parathyroid tetany. Attempts to apply this method in the treatment of human postoperative tetany have not been so successful.

The successful administration of viosterol intravenously to normal dogs in this laboratory suggested the trial of this method clinically.

* The expenses of this investigation were paid in part from a grant from Mead, Johnson and Co.

A woman, age 57, underwent a thyroidectomy and developed severe tetany within 2 days. During the next 6 months, the only treatment which consistently kept her free from tetany was calcium lactate by mouth with parathormone 2 or 3 times a week. Of the first she required approximately 115 gm. daily. Even this treatment, while maintaining her free from tetany did not render her normal. Full details of her clinical condition will be reported later.

After preliminary observations, viosterol was injected intravenously in 0.5 cc. amounts of 8000 D preparation in oil. No injurious effects were noted as a result of oil emboli. (Several hundred such injections have been made by Reed and Thacker[1] in dogs.)

TABLE I.

	Ca	Phos.	K.	B. M. R.	
Nov. 18	6.38	3.92			
" 20				35	
" 21				15	0.5 cc. viosterol i.v.
" 22	7.76	4.08	17.21		
" 23-25 inc.					20 gm. Ca lactate
" 25	3.98				0.5 cc. viosterol
" 26	8.69		15.19	16	0.5 cc. "
" 28	12.24	6.56	25.50		0.5 cc. "
" 29				7	0.5 cc. "
Dec. 1	10.08		20.88		
" 5	7.41*	5.03		3	
" 8	9.97	5.19			
" 9					1 cc. viosterol *per orum*, fasting
" 10					1 cc. viosterol *per orum*, 0.5 cc. i.v.
" 11					1 cc. viosterol *per orum*
" 12	8.88	6.92			1 cc. viosterol *per orum*

* Questionable determination.

We recognize the limitations in drawing conclusions from one case but the fact remains that for a period of 15 days the patient was kept free from tetany by the administration of a total of 2.5 cc. of 8000 D viosterol plus 20 gm. Ca lactate. Her general improvement is very striking. It is suggested that the failure of others to accomplish results clinically is due to the use of too small amounts of viosterol. The results of oral administration will be reported in full with other details.

[1] Reed, C. I., and Thacker, E. A., PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 187; *Am. J. Physiol.*, in press.

5326

Unmyelinated Sensory Fibers.

S. W. RANSON.

From the Institute of Neurology, Northwestern University Medical School.

Unmyelinated sensory fibers are present in large numbers in the spinal nerves. They take origin from the small cells of the spinal ganglia and divide like the myelinated fibers into central and peripheral branches.[1] The central branches enter the tract of Lissauer through the lateral division of the dorsal roots and probably end in the *substantia gelatinosa* Rolandi.[2] The peripheral branches run in the spinal nerves and are distributed chiefly in the cutaneous branches; very few are found in the motor nerves.[3] Ingvar has been able to confirm most of these observations on human material.[4]

In order to exclude the possibility of confusion due to the presence of sympathetic unmyelinated fibers the question has been reinvestigated on sympathectomized cats. The right abdominal sympathetic chain was removed from the diaphragm to the pelvis and 5 weeks or more allowed for the degeneration of the sympathetic fibers in the femoral nerve. Pyridine-silver preparations were made of the saphenous nerve and of the nerve to the *vastus medialis*. Great numbers of unmyelinated fibers were found in the saphenous nerve; but very few in the nerve to the *vastus medialis*. Other pieces of the same nerves from the same sympathectomized cats were treated with osmic acid. Counts showed that as many myelinated fibers could be seen in the pyridine silver as in the osmic acid preparations. In one fascicle of a saphenous nerve 1294 myelinated fibers were counted in the osmic acid preparation and 1258 in the pyridine silver preparation. It is obvious that when the unmyelinated fibers are taken into consideration the number of axons present in the sympathectomized saphenous nerve far exceed the number of myelin sheaths.

The unmyelinated axons stain much darker than the myelinated axons in pyridine silver preparations. They are closely grouped together in bundles. They must be sensory since there are no sympathetic fibers in these preparations, and very few if any unmyelinated fibers leave the spinal cord in the ventral roots.

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[1] Ranson, S. W., *J. Comp. Neurol.*, 1912, **22**, 159.

[2] Ranson, S. W., *J. Comp. Neurol.*, 1913, **23**, 259.

[3] Ranson, S. W., *Brain*, 1915, **38**, 381.

[4] Ingvar, S., *Acta Medica Scand.*, 1926-27, **65**, 645.

Unmyelinated fibers having exactly the same appearance are found in large numbers in the vagus[5] and splanchnic nerves.[6]

5327

Gall Bladder Visualization and Jaundice.

E. L. WALSH AND A. C. IVY.

From the Department of Physiology and Pharmacology, Northwestern University Medical School.

In this study we were interested in 2 questions: (1) Does tetraiodophenolphthalein when present in the gall bladder disappear following obstruction of the common bile duct on the ingestion of meals containing fat? (2) What is the effect of existing obstructive jaundice on the visualization of the gall bladder? To answer the first question the gall bladders of 5 dogs were visualized with tetraiodophenolphthalein, the common bile duct was ligated and meals containing fat were given daily. This is a repetition of the experiment of Copher.[1] It was found that the shadow becomes more dense for 2 or 3 days following the ligation of the common bile duct and this density is maintained as long as 2 weeks after the ligation. We did not follow any of the dogs for a longer period. These findings confirm those of Copher.

To answer the second question the common bile ducts of 5 dogs were ligated, and from 60 to 96 hours later the tetraiodophenolphthalein was injected. It was found that in 2 dogs the gall bladder was faintly perceptible in 14 hours, in one dog in 22 hours, and in the 2 others in 50 hours. At later periods up to 114 hours, the shadow slowly became more visible, but "normal density" was not obtained in any of the dogs. In each of these dogs the gall bladder was visualized and evacuated with egg yolk prior to common duct ligation. The presence of jaundice did not increase the toxicity of the dye.

It has been maintained by some writers[2] that the bile which enters the gall bladder is normally resorbed *in toto*. If this were true, it would be possible to claim that the disappearance of the gall bladder

[5] Chase, M. R., and Ranson, S. W., *J. Comp. Neurol.*, 1914, **24**, 31.

[6] Ranson, S. W., and Billingsley, P. R., *J. Comp. Neurol.*, 1918, **29**, 405.

[1] Copher, G. H., *J. Am. Med. Assn.*, 1925, **84**, 1563.

[2] Halpert, B., and Hanke, M. T., *Am. J. Physiol.*, 1929, **88**, 351.

shadow on the ingestion of a meal is due not to evacuation of the gall bladder, but to increased resorption of the dye. However, our results show that the ingestion of a number of meals with the common duct tied does not cause a decreased density nor a disappearance of the shadow. The only way to explain adequately the disappearance of the shadow in the dog 2 or 3 hours after a meal of egg yolk or within an hour after the injection of cholecystokinin is that most of the gall bladder contents leave via the biliary passages. In view of the mass of data in the literature on man, the cat and the dog showing that the gall bladder can evacuate, one cannot avoid accepting the foregoing explanation as a proven fact for these animals.

The results on the second question demonstrate that in obstructive jaundice in the dog the gall bladder does not visualize in a normal manner in that it is slow in visualizing and the density of the dye does not develop to the usual extent. This result is not surprising in view of the fact that the formation of bile is decreased in obstructive jaundice. Recently Rudisill[3] has found that the gall bladder of man may visualize in certain types of jaundice. Rudisill also found that in jaundice in man the toxicity of the dye is not increased, which we have confirmed in the dog.

5328

Protein Digestion in the Human Stomach.

D. H. WELKER AND O. BERGEIM.

From the Department of Physiological Chemistry, University of Illinois.

The primary function of the stomach being to digest protein, it would be desirable to be able to determine the actual accomplishment of the stomach in health and disease as far as protein digestion is concerned, instead of relying on acidity determinations as an indication. Determinations of the ratio of soluble to insoluble protein in gastric contents following a test meal might give useful information provided that the gastric contents remained a uniform suspension, and provided solid and liquid left the stomach at the same rate. On the other hand, if insoluble iron oxide were mixed with the flour and baked into the bread used as a test meal and if this oxide adhered to the undissolved portion of the gastric contents,

[3] Rudisill, H., *J. Am. Med. Assn.*, 1930, **95**, 1425.

the ratio of undissolved protein to iron would enable one to determine the percentage of protein of the test meal that had been put in solution by the gastric juice, and such a result would be fairly accurate whether or not there were stratification of gastric contents or selective evacuation of the stomach. This method should also provide a check on the significance of results of acidity determinations and of the use of the simple ratio of dissolved to undissolved protein in gastric contents.

Twenty-four normal men were given test meals consisting of 80 gm. of bread (made from flour containing 0.25% of red iron oxide) and 200 cc. of water. Twenty-five cc. portions of gastric contents were aspirated at 15 minute intervals (beginning at 30 minutes) until the stomach was empty. Determinations were made of dissolved protein (by centrifugation of contents and washing of the residue), of total protein, of undissolved protein, of iron, of pepsin and of free and total acidity. From this data the percentage of protein dissolved through gastric action was estimated from the ratio of dissolved to undissolved protein, and from the ratio of iron to undissolved protein. The percentage of protein dissolved ran in most cases from 60-90% with the highest values usually in the third specimen. Usually similar results were obtained by either method of calculation, but in certain cases the first method gave lower results, indicating that some stratification had occurred and that the protein:iron ratio was more dependable. Both methods were inaccurate when the stomach was practically empty. A general parallelism existed between the results on protein digestion and the acid and pepsin determinations. The iron ratio method appears to give the best estimates as to the effectiveness of the stomach in protein digestion and thus to be useful in checking other procedures. The chief difficulty in the way of expressing the protein digesting power of the stomach in a definite figure lay in the fact that the evacuation time of the stomach varies so much in normal and pathological conditions that analysis of the gastric contents at any selected time after ingestion of a test meal can not be depended upon to indicate the high point of gastric digestion in all cases. This is particularly true in cases of hypoacidity where gastric evacuation is often much hastened. This may be overcome by making analyses at intervals, but this makes the method at present too laborious for clinical use.

5329

Destruction of Yeast in the Normal Human Stomach.

B. E. MONTGOMERY, A. K. BOOR, LLOYD ARNOLD AND OLAF BERGEIM.

From the Laboratories of Physiological Chemistry and Bacteriology, University of Illinois College of Medicine.

Twelve gm. portions of bakers' yeast were fed to normal men with water or with various test meals. The stomachs were emptied after varying periods of time and counts of live yeast cells made by plating on malt agar. Microscopic counts of cells in gastric contents were also made. As no yeast cells are disintegrated in the period of normal gastric digestion, the percentage of yeast cells killed could be calculated from the 2 counts and the approximate proportion of yeast cells passing through the stomach alive under different conditions estimated.

When yeast was administered with each of the following the percentage of live cells getting through the stomach was approximately as follows: Water 60 cc., 95%. Water 250 cc., 90%. Orange juice 100 cc., 75%. Orange juice 250 cc., 55%. Yeast alone, 75%. Oatmeal gruel 500 cc., 95%. Milk 100 cc., 80%. Meat 100 gm., 50%.

Yeast went through the stomach most rapidly when taken with about 60 cc. of water (85% in 15 minutes), and most slowly when taken with meat (80% in 1½ hours).

In vitro experiments on human gastric juice indicated that the hydrochloric acid of the gastric juice was chiefly responsible for the destruction observed.

5330

Ventriculin in the Treatment of Pernicious Anemia Patients on Meat Free Diet.

ERNESTINE KANDEL. (Introduced by Louis Leiter.)

From the Medical Service of the Billings Memorial Hospital, and the Department of Medicine, University of Chicago.

After the demonstration by Castle[1] that the stomach of normal persons secretes a substance which can develop a blood maturing principle from meat, and the subsequent demonstration of ventricu-

[1] Castle, W. B., *Brit. Med. J.*, 1929, **1**, 1120.

lin as a hematopoietic stimulant by Sturgis and Isaacs,[2] it occurred to us that the action of ventriculin might be dependent in part on a muscle meat diet. The following 5 cases were therefore put on a meat free, high caloric diet 3 days before ventriculin was started, and kept on this diet during the experiment.

All 5 cases gave the classical history, physical findings, and blood picture of pernicious anemia. Standard tests were used throughout. The Sahli Hemoglobinometic was used for hemoglobin determinations. The presence of achlorhydria was determined by the histamine test. Platelets were counted by the indirect method.

Case I.—L. A., a man aged 49 years, entered the hospital after symptoms of 4 years' standing. He had had no previous treatment. Laboratory tests showed a hemoglobin of 35%. His initial red blood cell count was 1,260,000 per cubic millimeter; white cells, 5,250; platelets, 90,000. The reticulocyte count varied from 0.5% to 1% on 4 successive days. As we were interested in the potency of suprarenal extract as a hematopoietic stimulant, the patient was given 6 capsules of suprarenal extract daily before beginning the treatment with ventriculin. On the fourth day there was a beginning rise in reticulocytes, and on the ninth day a maximum of 9% was reached. There was a slight rise in the blood cells of 300,000, with a decrease on the sixth day after the reticulocyte peak which had now dropped to the original level of 1%. Suprarenal extract was then discontinued and the patient was given 3 vials (60 gm.) of ventriculin daily. On the second day the reticulocytes again numbered 9%, reaching a peak of 25.5% on the sixth day. There was a steady rise in the red blood cells and hemoglobin, with increase of platelets until on the twenty-third day the hemoglobin was 68% and the red blood cell count 3,600,000.

The clinical picture had improved steadily during this time, and the patient was discharged on treatment. At the end of 3 months the patient had a hemoglobin at 84%, red blood cell count 4,680,000, and he was looking for work.

Case II.—E. I., a woman aged 44 years, was admitted with symptoms of 3 years' duration. She had been on inadequate liver therapy, having been told to take the broth from a half pound of liver daily. The patient had repeated spells of syncope on examination and had been unable to retain food for several weeks. Initial hemoglobin was 30%, red blood count 1,300,000, white cell count

[2] Sturgis, C. C., and Isaacs, R., *J. Am. Med. Assn.*, 1929, **93**.

4,400, platelets 75,000 and reticulocytes 4%. The differential film was typical of pernicious anemia with a marked right shift in the

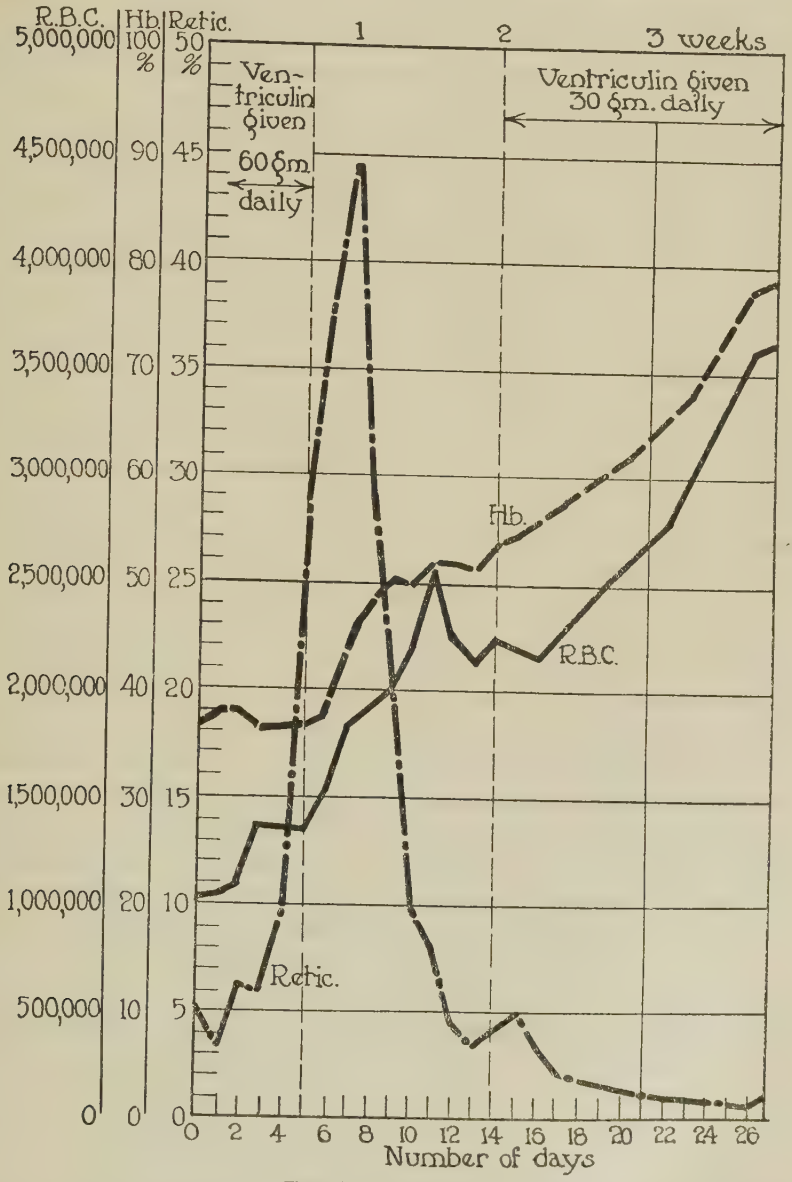

FIG. 1. CASE III.

Initial reticulocyte count 4-5%. 60 gm. of ventriculin given for 5 days. Maximum rise of reticulocytes to 44.5% after 6 days with rise in red blood cells and hemoglobin. On the 11th day the red blood cell count began to drop and reticulocytes were 4.5%. 40 gm. of ventriculin were again given daily with marked rise in red blood count and hemoglobin.

polymorphonuclear cells. Ventriculin was started after the 3 day meat free diet was begun. Owing to nausea and vomiting only 10 gm. (1 vial) was retained the first day. The next day 4 vials were given and most of it retained. The third day all 4 vials were retained and there was a slight rise in the reticulocytes. On the fifth day there was a 29% reticulocyte count, reaching a peak of 48.8% at the end of the sixth day. The reticulocyte count dropped abruptly with subsequent rise in hemoglobin and red blood cells to 70% and 3,480,000 respectively. There were great showers of platelets and a sharp rise in the white blood cells from 4,400 to 8,000.

On the sixteenth day the patient went home. Two weeks later she returned with a hemoglobin of 80% and a red blood count of 3,800,000. She walked briskly and remarked that she had not felt so well in years.

Case III.—E. I., a man aged 54, was admitted with a history of only 6 months' illness. History and physical findings were typical of pernicious anemia. The patient had not been treated previously.

The hemoglobin was 37%, red cell count 1,050,000, white cell count 4,500 and platelets, 70,000. The reticulocytes varied from 2% to 5.5%. Ventriculin was given in 60 gm. doses daily. On the third day the reticulocytes numbered 10%, and on the fourth day 29%. On the fifth day when the red blood cell count had reached 1,400,000 the ventriculin was stopped. This was done to see how long the effect of the ventriculin would last. At the end of the sixth day the reticulocyte count reached the peak of 44.2%; the red cells were 2,500,000 and the hemoglobin 52% on the ninth day. Seven days after the ventriculin was discontinued the red cell count began to go down, reaching 2,100,000. The reticulocytes had fallen abruptly to 4.8%. Ventriculin was again given in doses of 30 gm. daily at this point, with a rise of reticulocytes to 6% two days later. On the twenty-sixth day after treatment the hemoglobin was 79% and red blood cell count 3,620,000; platelets were 420,000. Clinical symptoms were markedly improved.

Case IV.—A. I., a woman of 63 years of age, was admitted after a year's illness, with a remission of 3 months' duration 3 months before admission to the hospital. The patient was mentally incompetent to describe previous treatment. Initial hemoglobin was 38%, red blood cells 1,600,000, white blood cells 3,800, reticulocytes 2%. The patient was given 40 gms. of ventriculin daily. Owing to the author's enforced absence from the work, daily counts were interrupted for a week. On the seventh day the reticulocyte count was 11% and on the following day 17%. It was assumed that this

was the peak of the reticulocyte count, but this is not necessarily true. However, there was a slow but steady rise of hemoglobin and red blood cells, and on the twenty-sixth day after treatment they were 72% and 3,920,000 respectively.

Case V.—J. C., a woman aged 62, entered the hospital after an illness of 3 years' duration. There was an indefinite history of irregular liver therapy. The patient was in a highly irritable state and the marked mental improvement on treatment was spectacular. Initial hemoglobin was 49%, red blood cells 1,500,000, white blood cells 4,820 and reticulocytes 4%. The patient was given 4 vials of ventriculin daily. As there was no increase in reticulocytes on the fifth day, and as the patient was becoming very hard to manage, 6 vials were given daily with an increase of reticulocytes to 13.5% the following day. Unlike the preceding cases which showed a marked rise and fall in reticulocytes, the percentage remained between 13% and 16% for a period of 8 days, and then gradually dropped.

In 3 weeks the hemoglobin had increased to 70% and the red blood cell count to 3,490,000, and the patient was dismissed. She returned to the outpatient department in 2 weeks with a hemoglobin of 85% and a red blood cell count of 4,350,000.

Discussion.—There is little doubt even after the demonstration of only 5 cases that ventriculin does not depend on the addition of muscle meat in the diet for its stimulating action on the hematopoietic system. The reticulocyte rise was reached in most cases within the same time as it was reached in those patients who were given ventriculin with a meat diet as demonstrated by most other workers. This is particularly true of cases II and III. The stimulation effect of 240 gm. of ventriculin given over a period of 4 days would seem to be something over 8 days in one case. It would be interesting to know whether this coincides with the results of large doses of liver extract given in one or 2 doses.

Conclusion.—1. Ventriculin gives the typical hemopoietic response in the treatment of pernicious anemia patients on meat free diet.

5331

Food Poisoning Probably Caused by Orange Colored Staphylococcus from Udders of Apparently Healthy Cows.

R. J. RAMSEY AND P. H. TRACY. (Introduced by F. W. Tanner.)

From the Dairy Department, University of Illinois, Urbana, Ill.

While studying the cause of a malt flavor in raw milk, one of the authors, (R. J. R.) became ill with a severe gastroenteritis. He was prostrate for 2 days with severe symptoms of food poisoning, which included cramping in abdominal regions, weakness of legs, violent diarrhoea, and absolute loss of appetite.

The suspected food was raw milk which had been inoculated with a pure culture of an orange colored staphylococcus, isolated from a sample of raw milk delivered to a commercial milk plant. The authors did not suspect that this organism might be harmful, because of its common occurrence in raw milk delivered to the milk plant.[1] Small portions of the inoculated milk were consumed each day for 5 days by (R. J. R.) before his illness. This orange colored staphylococcus has been found to attack the casein of milk with the formation of a malt-like flavor and odor. In order to check the symptoms reported above, 4 kittens were fed milk cultures of this organism. In less than one week, 2 of the kittens had bloody stools, one having diarrhoea and the other having considerable mucous in the stool. The 2 remaining kittens had constant diarrhoeal stools until the cultured milk was omitted from the diet.

Our data seem to be in accord with many which have been recently reported. Barber[2] found a toxin-producing staphylococcus to be responsible for many attacks of gastroenteritis. This organism occurred in the udder of an apparently normal cow. Dack[3] traced an epidemic of food poisoning to a similar organism. The incriminated food was a Christmas cake in this case. Jordon[4] has recently published data showing intestinal disturbances to be due to a yellow, toxin-producing staphylococcus.

In order to establish the identity of the orange colored staphylococcus in question, it was compared with pure cultures of *Staphylococcus aureus*. Since the pure cultures of *Staphylococcus aureus* were found to produce a malt flavor in milk, and since they were

[1] Unpublished data, University of Illinois Dairy Department.

[2] Barber, *Phil. J. Science*, **9**, 515.

[3] Dack, Cary, Woolpert, and Wiggers, *J. Prev. Med.*, 1930, **4**, 167.

[4] Jordon, E. O., *J. Am. Med. Assn.*, 1930, **94**, 1648.

very similar in physiological characteristics to the incriminated organism, it is believed that the organism in question is also *Staphylococcus aureus*. Further work is in progress on the heat resistance of the toxic agent.

Iowa Section.

Iowa State College, Ames, Iowa, December 4, 1930.

5332

Rôle of Copper in Hemoglobin Formation.

H. L. KEIL AND V. E. NELSON.

From the Laboratories of Physiological Chemistry, Iowa State College.

The hemoglobin value of the blood of rats on our growing ration varies from 15 to 18%. The hemoglobin content of the blood of rats on whole milk falls to 2 to 5% in 10 weeks. The females respond to anemia and die sooner than the males. Ferric chloride increased the hemoglobin to 16% in 9 weeks. The ferric chloride was made from high grade standardization iron wire. The wire was dissolved in pure HCl to which was added a small amount of pure HNO_3, and H_2S was bubbled through the diluted solution to precipitate any copper present. The chloride was then crystallized. The Hilger spectrograph showed no copper in the ferric chloride or ferric chloride solution. Ferric chloride from electrolytic iron caused hemoglobin regeneration so that in 9 weeks a value of 16% was obtained. The electrolytic iron, $FeCl_3$, and $FeCl_3$ solution were copper free as shown by the Hilger spectrograph. If $CuSO_4$ be given with the ferric chloride, regeneration is quicker and requires 4 weeks to reach a value of 2.2% higher than without copper.

Rats in glass cages with glass screens did not become anemic any sooner than rats in cages with galvanized screens. Rats on shavings did not develop anemia in 20 weeks. Anemic rats were given sucrose dissolved in copper free water. One lot received in addition pure $FeCl_3$, another lot received 3.0 mg. copper as $CuSO_4$ per rat daily, and the third lot received sucrose and distilled water. The $FeCl_3$ lot had a hemoglobin value of 15% in 3 weeks. The $CuSO_4$ lot showed a slight gain in hemoglobin, due to a small amount of iron in the c.p. $CuSO_4$. The control lot showed no gain in hemoglobin, so after 2 weeks each rat was given daily 0.2 mg. of Fe as $FeCl_3$; and in 2 weeks the hemoglobin increased from 5 to 12%.

Manganese (0.1 mg. Mn per rat daily) as $MnCl_2$ when fed with $FeCl_3$ to anemic rats did not increase the value of hemoglobin above the value obtained on iron alone. Rats on whole milk, wheat embryo oil, and $FeCl_3$ did not reproduce. Copper sulphate addition to the above ration resulted in normal reproduction—3 females producing 9 litters of 59 young in 109 days. Lactation was fair. The milk used analyzed between 0.35 and 0.44 mg. Cu per liter. The cane sugar contained no copper. Unless otherwise indicated the $FeCl_3$ was fed at a level of 0.5 mg. Fe and the $CuSO_4$ was fed at a 0.05 mg. Cu level.

5333

Cod Liver Oil for Reproduction and Lactation.

H. O. SMITH AND V. E. NELSON.

From the Laboratories of Physiological Chemistry, Iowa State College.

Previous work in this laboratory has emphasized the fact that cod liver oil contains vitamin E. Some investigators have not had success with cod liver oil as a source of this vitamin, so it was deemed advisable to test different cod liver oils for their potency in the reproductive vitamin. The rations consisted of casein 18.0, salt mixture 185, 3.7, yeast 12, different cod liver oils from 1 to 5, and dextrin to 100%. Eight cod liver oils were examined. The growing ration served as a control and was outstandingly the best of the group. The growing ration gave a value of 5.4, whereas the oils gave the following figures: 1.79, 1.12, 0.88, 0.73, 0.72, 0.66, 0.56, and 0.08. The figures represent the number of young produced per female per month on each ration. Some of the oils contain far more vitamin E than others, although they contain less than some of our natural foods.

The mortality of the young varied with the kind of oil and the level at which administered. Five percent of one oil gave a mortality of 18%, while the same amount of another oil gave a mortality of 100%. The mortality with the first oil was no higher than on the growing ration. A 3% level of the better oil mentioned above gave a mortality of 9%, whereas the other oil gave a mortality of 70%. A considerable number of females died in pregnancy on the various cod liver oil rations. The mortalities of the females in pregnancy on the different oils, expressed as percent, were: 14, 5, 43, 36, 17, 19, 4, and 39. There appeared to be no relation between the mortality of the female and the potency of the oil in vitamin E.

Missouri Section.

St. Louis University School of Medicine, December 10, 1930.

5334

Effect of Intraperitoneal Injections of Potassium Iodide on Proliferative Activity of Thyroid Gland in Rats.

JACOB RABINOVITCH. (Introduced by L. Loeb.)

From the Department of Pathology, Washington University School of Medicine.

It has been shown that contrary to the action of thyroid substance, administration of potassium iodide to guinea pigs in which the greater part of the thyroid gland had previously been extirpated, does not prevent the occurrence of compensatory hypertrophy and that in normal guinea pigs it has a noticeably stimulating effect on this organ. It also produces a slight increase in the size of the epithelium, a certain degree of softening of the colloid and an increase in the number of phagocytes in the colloid.[1-9] Furthermore, the degree of cell proliferation and the intensity of the histological changes in the gland vary in accordance with the amount of iodide given, and the length of time during which it is administered, is also of importance.

Irsigler[10] has shown that very similar changes take place also in the thyroid gland of white rats when treated with potassium iodide.

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[1] Loeb, Leo, *J. Med. Res.*, 1920, **41**, 481; *Am. J. Pathol.*, 1926, **2**, 19; 1929, **5**, 79; *Endocrinology*, 1929, **12**, 49

[2] Gray, S. H., Haven, F. L., and Loeb, Leo, Proc. Soc. Exp. Biol. and Med., 1927, **24**, 503.

[3] Gray, S. H., and Loeb, Leo, *Am. J. Path.*, 1928, **4**, 257.

[4] Rabinovitch, J., *Am. J. Path.*, 1928, **4**, 601; 1929, **5**, 87 and 91.

[5] Gray, S. H., and Rabinovitch, J., Proc. Soc. Exp. Biol. and Med., 1929, **26**, 468.

[6] Rabinovitch, J., and Gray, S. H., *Am. J. Path.*, 1930, **6**, 75.

[7] McCordock, H. A., *Am. J. Path.*, 1929, **5**, 171.

[8] Silberberg, Martin, *Krankheitsforschung*, 1930, **8**, 171.

[9] Loeb, Leo, *Am. J. Surg.*, 1929, **7**, 12.

[10] Irsigler, F. H., *Beitr. z. Path. Anat.*, 1930, **85**, 221.

However, the amount of iodide necessary to produce these changes in the rat was considerably smaller than that used in the guinea pig; moreover, when somewhat larger doses were administered for the same or for longer periods of time, the increase in mitotic activity of the acinar epithelium was either diminished or completely prevented. As to the intensity of the mitotic proliferation produced by the administration of iodine, Irsigler makes only general statements without giving any definite figures comparable to those in the case of guinea pigs in the investigations from this laboratory.

Chouke[11] showed that when doses of potassium iodide corresponding by weight approximately to those which have been found active in the case of guinea pigs, or somewhat higher doses, are given to rats they do not produce an increased activity of the thyroid gland. Not only were the doses given greater but the time of examination was somewhat later than in Irsigler's investigations.

It was of interest to determine (1) whether the differences in the results obtained by these 2 investigators were due to the difference in the dosage and in the time of examination, and (2) to supply exact data as to the increase in mitotic activity caused by KI in rats. Twenty-four white rats with weights varying approximately between 120 and 190 gm. were divided into 4 sets. Each animal in the first set received daily injections of 0.0005 gm. KI dissolved in 1 cc. of sterile distilled water; the animals of the second set were similarly injected with 0.002 gm. KI and those of the third set received 0.01 gm. KI. The fourth set, serving as controls, did not receive KI. The injections were given in each set for periods of 5, 10 and 15 days respectively. At the end of each period the animals were killed by chloroform, the thyroids removed and studied in the same manner described in previous reports.

Table I indicates the number of mitoses observed in the various

TABLE I.—MITOSES.*

Time days	Control		0.0005 gm. KI		0.002 gm. KI		0.01 gm. KI	
5	0	22+	5472	30—	2980	0	200	15—
5	0	10—	4300	25—	2354	0	285	38+
10	48	30+	954	10+	350	40—		
10	0	6+	380	0	688	5+	150	10+
15	36	40+	300	95+	2124	13+	104	0
15	24	20+	180	0	100	10—	100	0

* + gain in weight; — loss in weight.

[11] Chouke, K. S., *Endocrinol.*, 1930, **14**, 169.

animals under the different experimental conditions. The highest proliferation activity is found in animals that have received the smallest amount of KI for the shortest period of time, namely 0.0005 gm. for a period of 5 days. As the amount of iodide is increased and the period of time is lengthened, there follows a corresponding reduction in the number of mitoses. Accordingly, we find in animals that have received the largest dose of KI for the longest period of time, namely 0.01 gm. KI for 15 days, the smallest number of mitoses. Occasional individual variations are noted; the animal that received 0.002 gm. KI for 15 days had 2124 mitoses while the other animals that received a similar small amount of KI had a markedly lower mitotic count. Occasional variations of this kind merely indicate that the thyroids of different animals respond with somewhat varying intensity to the stimulating effects of potassium iodide. If we consider, however, the experimental results obtained as a whole, there remains no doubt that KI exerts a marked stimulation on the thyroid gland of the rat and causes an increased proliferative activity when used in appropriate amounts. In this respect therefore, our experiments agree with the results obtained by Irsigler, and we feel that the cause of the different results obtained by Chouke and Irsigler are due to the difference in doses of KI and also in the periods during which it acted in the experiments of these 2 investigators.

In addition to the increased mitotic proliferation induced by suitable doses of KI, there were other microscopic changes in the thyroid gland likewise indicative of stimulation. Reference has already been made to such changes which briefly consist of a moderate softening of the colloid, an increase in the number of phagocytes in the colloid as well as a slight increase in the height of the acinar epithelium. In the present series of experiments these changes were also found and were most definite where the increase in proliferative activity was most marked. Where this increase was lacking or very slight, the thyroid retained its normal characteristics; the colloid tended to remain solid, the number of phagocytes was relatively small and the epithelium was low columnar or cuboidal. In some cases the diminished number of mitoses was associated with a distension of the acini due to an accumulation of colloid, and almost complete flattening of the lining epithelium. The latter changes are very similar to those previously observed by Gray and Loeb and by ourselves in guinea pigs receiving potassium iodide over a very long period of time.

Definite changes in body weight in the individual animals during

these experiments did not seem to be associated with the changes in cell proliferation. In previous investigations, on the other hand, it was noted[4, 6] that a considerable loss in body weight in the animal interfered with the stimulating effects of potassium iodide and caused a diminution in gland activity. In our experiments with rats 2 of the animals that showed the largest mitotic count suffered from a moderate loss in body weight. It is quite possible that this loss of weight was secondary to the heightened activity of the thyroid gland resulting from the stimulating effects of the iodide.

Conclusions. Our experiments tend to reconcile the differences between the results obtained by Chouke and Irsigler, and our results confirm those of Irsigler as to the necessity of using relatively small doses and short periods of time in order to obtain stimulating effects on the thyroid of the rat by the administration of KI. There seems to exist a quantitative relation between the increase in mitotic proliferation and the quantity of KI used and the length of time during which it acted. In the case of the guinea pig we have shown previously that within the limits tested the larger doses are more effective than the smaller ones. Whether a similar relation exists also in the case of the rat thyroid remains to be seen. It is of interest that the maximum number of mitoses observed in the rat under the influence of optimal conditions of stimulation is very similar to that formerly established in the case of the guinea pig.

Associated with this increased proliferative activity of the acinar epithelium, there are observed the other structural changes in the thyroid gland indicative of increased metabolic activity, previously noted in the case of the guinea pig. The average index of mitotic proliferation which we found in the thyroid gland in the control rats is somewhat lower than that observed by Chouke. This may possibly be due to differences in the season of the year; however, this point needs further investigation.

5335

Studies on Bacterium Granulosis in Relation to Trachoma: Pathogenicity for Various Monkeys and Apes.*

CHARLES WEISS.

From the Department of Ophthalmology, Washington University School of Medicine, St. Louis.†

Noguchi's discovery[1] of a bacterium which can be artificially cultivated and which produces granular lesions in the conjunctivae of monkeys has supplied a new approach in the field of trachoma investigation. Noguchi, Tilden and Tyler,[2] working with rhesus monkeys succeeded in inducing active, progressive lesions which lasted 8 or more months in the conjunctivae of 19% of a total of 159 rhesus monkeys, and only transient lesions (granulations which receded before the 8th month) in an additional 34.5%. Their results with 10 chimpanzees were as follows: Progressive lesions lasting 8 months or longer in 30%, and transient lesions in 50%. Two out of the 10 chimpanzees failed to react.

These statistics compare favorably with the findings of previous investigators working with fresh trachoma "virus"‡ obtained directly from trachomatous lesions. However, the long periods of incubation (up to 150 days), reported in the above studies, are in striking contrast to the much shorter periods (2 to 20 days) observed by other writers in experiments on both human subjects and various species of monkeys.[3, 4, 5, 6, 7]

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* This paper will appear in full in the *Transactions of the American Academy of Ophthalmology and Otolaryngology*, 1930.

† Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] Noguchi, H., *J. Exp. Med.*, 1928, **48**, Supplement 2.

[2] Tilden, E., and Tyler, J., *J. Exp. Med.*, 1930, **52**, 617.

‡ The term "virus" is here used in the French sense, signifying the infectious agent as contained in material removed from human lesions, without reference to filterability.

[3] Taborisky, J., *Arch. f. Ophth.*, 1929, **123**, 140.

[4] Halberstaedter, L., and Prowazek, S. von, *Deutsche med. Wchnschr.*, 1907, **33**, 1285.

[5] Greeff, R., Frosch, H., and Clausen, W., *Arch. f. Augenh.*, 1907, **58**, 52; 1908, **59**, 203.

[6] Nicolle, C., Cuénod, A., and Blaizot, L., *Arch. de l'Inst. Pasteur de Tunis, Fascic.*, 1913, **3**, 157. Nicolle, C., and Cuénod, A., *Arch. Inst. Pasteur de l'Afrique du Nord*, 1921, **1**, 149.

[7] Hess, C., and Römer, P., *Arch. f. Augenh.*, 1906, **55**, 1.

We§ have repeated some phases of Noguchi's work with 3 strains of *B. granulosis,* furnished by the Rockefeller Institute, and later with 2 others isolated by ourselves at the Pasteur Institute of Tunis and at Washington University. We employed 28 rhesus monkeys, 2 *M. inuus,* 1 baboon, and 1 callitriche.

Noguchi's technic as amended in the notes kindly sent by Dr. Flexner was followed. Olitzky's technic[8] of massaging the lids was also used in the latter part of the work.

We cite briefly 2 protocols as examples.

Inoculation with *B. granulosis* (Strain Tunis 34). *M. inuus* (Algerian magot) injected July 4, 1929, into the right eye with a freshly isolated culture. 16th day: both eyes showed a few, fine, granulations in the cul-de-sac and on the superior tarsi. Inflammation at the internal angle and marked edema of the lids. No secretion. 33rd day: both eyes showed persisting granulations on the superior and inferior palpebral conjunctivae. These lesions began to regress on the 50th day and had disappeared on the 90th day. Another magot similarly injected produced essentially the same results.

Inoculation of *B. granulosis* (Rockefeller Culture "A"). *M. rhesus,* injected April 30, 1929, with material from the original ampoule. 10th day: edema and hyperemia at the site of the injection. The 71st day showed a ridge of 3-4 follicles on the superior margin of the tarsus, with hyperemia. 78th day: the uninjected eye had 2 small follicles on the tarsal plate. Both eyes were clear on the 167th day.

An examination of Table I shows that various strains of *B. granulosis* induced in a certain percentage of monkeys a type of granular conjunctivitis similar to that described by Noguchi. The lesions in our animals were, however, all of the mild and transient type; *i. e.,* never very extensive and never persisting more than 4 months. Varying degrees of edema and inflammation accompanied these follicles or granulations, but no thickening nor loss of transparency of the conjunctivae, nor evidence of secretion were observed.

In 2 young chimpanzees inoculated with the Rockefeller cultures only transient follicles were induced, whereas in a third, chimpanzee infected with fresh "virus" obtained from human trachomatous eyes, advanced lesions developed which contained Prowazek bodies

§ We wish to acknowledge our appreciation of the kindness of Parke-Davis & Company and of Eli Lilly & Company, who supplied the horse blood and serum used in the preparation of our culture media.

[8] Olitsky, P., *Rev. intern. du Trachome,* 1930, 7, 173.

TABLE I.

Results of Injection of Various Monkeys and Chimpanzees with Cultures of B. Granulosis.

Strain of *B. granulosis* Injected	Species of Animals Used	No. Injections	Animals with Negative Results	Animals with Transient Lesions Persisting to 4 mo. (Receded)
Rockefeller "A"	*M. rhesus**	3	2	1
	Baboon	1	0	1
	Callitriche	1	1	0
Rockefeller "B"	*M. rhesus*†	11	8	3
	Baboon	1	0	1
	Chimpanzee	2	0	2
Rockefeller M. C.	*M. rhesus*	5	4	1
	Chimpanzee	1	1	0
Tunis 34	*M. rhesus**	5	5	0
	Baboon	1	1	0
	M. inuus	2	0	2
	Chimpanzee	1	0	1
St. Louis 13 L	*M. rhesus*	5	2	3
	Chimpanzee	1	0	1
Denver 7‡	*M. rhesus*	3	3	0
	M. rhesus	32	24	8 or 25 %§
	Baboon	3	1	2 or 66.6 "
	M. inuus	2	0	2 or 100.0 "
	Chimpanzee	5	1	4 or 80.0 "
	Callitriche	1	1	0 or 0.0 "

Total Number of Animals Used.

28 *M. rhesus* (3 reinjected); 1 Baboon (injected 3 times); 2 *M. inuus;* 2 Chimpanzees (1 injected twice, the other 3 times); 1 Callitriche.

* Infection attempted twice.

† Infection attempted 3 times.

‡ Furnished by Drs. Finnoff and Thygeson.[9]

§ Calculated on the basis of total number of attempts at infection.

and both clinically and histologically showed a striking resemblance to the lesions of an active human trachoma. Thus 18 days after infection, the mucous membranes of the inferior conjunctiva were thrown into folds, there were papillary hyperplasia and hypertrophy, secretion and ptosis of the upper lid. The infection spread spontaneously to the uninoculated eye and follicles developed in the upper and lower conjunctivae of both eyes including the tarsi and retrotarsal folds. Neither in the material removed from the conjunctival lesions of the patient nor from those of the chimpanzee were we able to isolate *B. granulosis.*

Histological sections of the conjunctivae of the inoculated eye of this champanzee taken on the 216th day after injection (when the animal died of generalized tuberculosis) showed epithelial invagina-

[9] Finoff, W., and Thygeson, P., Presessional Volume, Section on Ophthalmology, American Medical Association, Detroit Meeting, June 23-27, 1930.

tions, lymphocytic infiltration, young fibroblasts and new connective tissue containing many small capillaries. Near the upper end of the tarsus, the layer of infiltrated inflammatory cells was thicker and contained plasma cells and histiocytes. The lids of the uninoculated eye showed a similar picture. The centers of the follicles contained groups of epithelioid cells.**

5336

Oxidation of Lactate by Methemoglobin in Erythrocytes with Regeneration of Hemoglobin.

W. B. WENDEL. (Introduced by P. A. Shaffer.)

From the Laboratory of Biological Chemistry, Washington University School of Medicine.

In earlier communications[1] we reported the oxidation of lactic to pyruvic acid and hemoglobin to methemoglobin by normal dog erythrocytes in the presence of 0.005% methylene blue. Pyruvic acid is not further oxidized. Because the oxygen partition seemed to indicate coupled reactions, it was tentatively suggested that the mechanism was one of peroxidation. Further studies, in which the stochiometric relationships observed with sugar-free cells were not obtained in presence of glucose, led us to question the explanation first offered.

Warburg, Kubowitz and Christian[2] explain methylene blue catalysis in red blood cells by the following chain of reactions: (a) oxidation of hemoglobin to methemoglobin by methylene blue; (b) oxidation of carbohydrate (or derivative) by methemoglobin with regeneration of hemoglobin; (c) oxidation of leuco-methylene blue by O_2. (A detailed paper by Warburg *et al*[3] which reached us while writing this report appears to substantiate their earlier interpretation.)

If methemoglobin is the agent responsible for methylene blue catalysis in red blood cells, it should be possible to oxidize lactic acid with methemoglobinized cells in the absence of O_2. And if no

** We are indebted to Dr. Harvey D. Lamb for the histological report on this specimen.

1 Wendel, PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 865; 1930, **27**, 624; *J. Biol. Chem.*, 1930, **87**, p. xx.

2 Warburg, Kubowitz and Christian, *Biochem. Z.*, 1930, **221**, 494.

3 Warburg, Kubowitz and Christian, *Biochem. Z.*, 1930, **227**, 245.

other oxidations occur, an equivalent amount of hemoglobin should be formed, as follows:

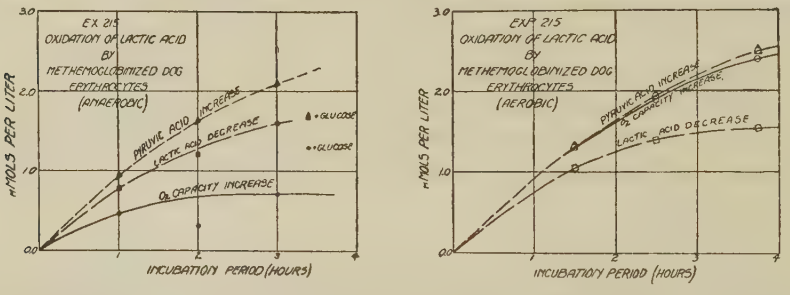
I. $2\,CH_3 \cdot CHOH \cdot COOH + MetHb(Fe^{+++})_4 \rightarrow 2\,CH_3 \cdot CO \cdot COOH + Hb(Fe^{++})_4 + 4H^+$.

and on exposure to oxygen,

II. $Hb(Fe^{++})_4 + 4O_2 \rightarrow Hb(FeO_2)_4$

The equations say that for each mol of pyruvic acid formed 2 mols of O_2 capacity are created. (This would be the minimum ratio; if other oxidations occur simultaneously, a larger reduction of methemoglobin should be found.) Furthermore the increase in O_2 capacity should be the same whether reaction I. occurs in presence or absence of O_2.

Experiment: Dog erythrocytes, treated with amyl nitrite until all hemoglobin is converted to methemoglobin (as measured by O_2 capacity), washed twice with saline to remove excess nitrite, were suspended in isotonic Na lactate-phosphate-chloride solution (pH 7.47), and incubated (37.5°) with rocking in wide bottom flasks (aerobic) and also in closed filled tubes (anaerobic). At intervals samples were analyzed for lactic acid, pyruvic acid and O_2 capacity.



The figures illustrate the results obtained. The rate of pyruvic acid formation is the same aerobically and anaerobically. The increase in O_2 capacity is greater aerobically than anaerobically; but in neither case is it enough to account for the pyruvic acid formed. (The difference between pyruvic acid increase and lactic acid decrease is probably due to progressive decomposition of a hexose-phosphoric ester with formation of lactic acid.) With both glucose and lactate present, the rate of methemoglobin reduction is increased and lactate oxidation *reduced,* indicating preferential oxidation of glucose. This behavior probably indicates the manner in which methemoglobin, formed in red cells by drugs or from other causes, is in the living animal reconverted to hemoglobin, and may also account for the apparent difficulty in producing methemoglobin in blood of some species.

The discrepancy between methemoglobin reduced (increase of O_2 capacity) and lactate oxidized means either that the nitrite-"methemoglobin" is not methemoglobin (Hartridge[4]) and has much higher capacity as an oxidant, or that other oxidants are involved beside methemoglobin. The question is being investigated.

Methemoglobin in simple buffer solution (in absence of cells) fails to attack lactic acid, and methemoglobin dissolved in buffer solution, in which normal hemoglobin-containing, glycolytically active cells are suspended, likewise fails to oxidize lactate. We may infer, therefore, that methemoglobin is effective in causing this oxidation only when present within the cell where lactate activation occurs.

5337

Ovarian and Anterior Pituitary Hormones from the Pregnant Monkey.

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From the Department of Anatomy, University of Missouri.

The hormones referred to are (1) the ovarian hormone (theelin, oestrin, folliculin) and (2) the active principle of the anterior lobe of the hypophysis which stimulates development of ovarian follicles and culminates in ovulation. The simplest and most decisive test for the former is the full oestrous growth reaction of the epithelium of the genital tract of the ovariectomized adult rat determined by the characteristic changes in the cell content of vaginal smear preparations. One of the best tests for the second active substance is the reaction of the normal immature rat which results in the attainment of puberty including the first ovulation. This is accomplished by stimulation of rapid growth in the ovarian follicles which in turn produce the follicular hormone which causes rapid growth in the genital organs.

It has been demonstrated repeatedly that the human placenta contains large amounts of folliculin, the total increasing with the growth of the placenta as pregnancy progresses. Positive tests have been obtained from fetal membranes of the cow, sheep and horse but negative tests so far from zonular placentas of the cat and dog. The urine of pregnant women and cows furnishes an abundant

[4] Hartridge, *J. Physiol.*, 1920-21, **54**, 253.

source of this hormone. The gonad stimulating principle of the anterior pituitary is also present in human urine during pregnancy.

We have tested 2 full term placentas of the monkey, *Macacus rhesus,* for ovarian hormone and the urine of one pregnant monkey for both this and the anterior pituitary hormone.

As shown by Hartman, the gestation period in monkeys is approximately 6 months long. The placentas used for these tests were obtained by Dr. Hartman from 2 of the monkeys photographed by him during parturition. They were preserved in alcohol and sent us for extraction. The residue from the alcoholic extract was dissolved in Mazola oil and tested in ovariectomized adult rats. The first tests of these extracts in moderate doses were negative. Later, with increased doses, positive tests were obtained. The number of these tests was not great enough to justify an exact quantitative statement. It was sufficient, however, to demonstrate the presence of small amounts of oestrin in monkey placenta.

One female in our colony mated between the 9th and 12th of December, 1929, became pregnant. Pregnancy continued until the 19th of April, 1930, at which time a normal fetus was aborted. Overnight samples of urine were collected from this monkey at intervals beginning in January and continuing until the time of abortion.

Tests for the gonad stimulating anterior lobe hormone were made by injecting urine directly into normal immature rats aged 24 to 29 days at the beginning of injections. Twelve tests were obtained of samples collected between January 24th and April 16th. Total dosages ranged from 3 to 8 cc. of urine over periods of several days. The ovaries were examined immediately after removal, then sectioned and studied microscopically. In no case was a positive result obtained from urine of this monkey although smaller amounts of urine from pregnant women returned positive reactions in control experiments. In the urine from this animal, therefore, there was very little if any of this anterior lobe substance.

Parts of the same samples of urine were extracted with chloroform, the residue dissolved in Mazola oil and tested for folliculin. In moderate dosages these extracts proved negative, but when the dosages were increased, positive results were obtained. It was concluded that this hormone is present in the urine from the pregnant monkey but in less concentration than in the urine from pregnant women.

Pacific Coast Section.

Stanford University, December 23, 1930.

5338

Colloidal Gold Test for Detection and Titration of Immune Bodies in Poliomyelitis.*

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The test depends upon the precipitation of a colloidal gold sol by an electrolyte (sodium chloride) in the presence of serum containing varying amounts of immune bodies specific for poliomyelitis.

Preliminary experiments were made with normal and immune monkey and normal and poliomyelitic human serums in the presence of varying dilutions of Kolmer's cholesterinized antigen and different concentrations of sodium chloride. Other antigens such as plain and cholesterinized extracts of normal rabbit and sheep brain were studied also.

In a manner similar to that described by Mishulow and Krumwiede,[1] Sanderson and Yoe,[2] for the determination of toxicity of diphtheria toxin, and recently by Jungeblut[3] in poliomyelitis, an investigation was made of the possible effect of 1% gold chloride. The results were not specific or consistent and the use of antigens and gold chloride was discarded. A peculiar color change ranging from lilac to deep purple was observed in certain tubes that contained immune serum in the presence of this reagent, however, and the idea of a color reaction suggested itself. Inasmuch as the antigens and gold chloride had seemed to possess no particular merit in such a test, Lange's colloidal gold was used.

The method adopted finally utilized colloidal gold, sodium chlo-

* Work done under a grant for poliomyelitis research, Mount Zion Hospital, San Francisco.

1 Mishulow and Krumwiede, *J. Immunol.*, 1927, **14**, 77.

2 Sanderson and Yoe, *J. Immunol.*, 1929, **16**, 427.

3 Jungeblut, C. W., Proc. Soc. Exp. Biol. and Med., 1930, **28**, 7.

ride (0.4%) and serum in proportions that were found most effective and clear cut in numerous preliminary determinations. Dilutions of serum were prepared in series in an attempt to detect quantitative differences. Readings were made after 12-16 hours. With cholesterinized sodium chloride, however, results could be read easily after 3 hours at room temperature (22-25°C.).

Composite experiments were repeated with uniform agreement and the results appeared decisive. Poliomyelitic serum from convalescent adult and juvenile patients and immune monkeys consistently produced a rose-colored or violet precipitate with a supernatant fluid graded from clear and colorless to light salmon pink and finally the negative rose color of the control. Most marked precipitation occurred in the tubes containing the lower dilutions of serum. Normal monkey serum and that from normal children in the susceptible age groups (2-7 years) were negative for precipitation effect, as were the serums from convalescent typhoid fever patients and from rabbits immunized with different strains of streptococci and staphylococci. Further studies are in progress with potent typhoid and paratyphoid serums and with antimeningococcus, antipneumococcus, and antitetanus serums.

In a few instances serum from supposedly normal adults showed very slight color changes in the high concentrations of serum and rarely a faint precipitating effect. This observation confirmed the impression that adults may have immune bodies for poliomyelitis as a result of a previous undetected mild attack of the disease or exposure to it.[4]

Positive Wasserman and Kahn reactions in serums did not affect the specificity of the colloidal gold test for poliomyelitis immune bodies.

The pH value of the mixtures used in the test showed that this was not a factor. The controls included a wide range of buffer solutions and the test serums before and after reading the reactions with colloidal gold and electrolyte.

The effect of combining poliomyelitis virus filtrate as a serum diluent before addition of the gold sol and electrolyte was to prevent the precipitation. Such serums behaved like that of the untreated normal monkey and suggested an absorption phenomenon. Other filtrable viruses are being tested in this manner to verify this possibility.

A preliminary application of the colloidal gold test in the study of antibody formation during the course of poliomyelitis was made

[4] Shaughnessy, *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 742.

in experimentally infected *M. rhesus* monkeys. Serum was tested at intervals prior to the appearance of the earliest signs of paralysis or symptoms, and up to the third day of paralysis, with negative results. Further studies are in progress along this line of investigation and in connection with the development of quantitative procedures.

The correlation of immune body content and protective property of serums is also being studied *in vitro* and *in vivo* in monkeys, and serums from all possible sources are in the course of investigation.

5339

A Rapid Method for the Diagnosis of Early Pregnancy from Urine.

FREDERICK EBERSON.

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The method is based upon a simple procedure for concentrating the anterior pituitary hormone[1] in the urine and injecting 2 or 3 small doses subcutaneously into immature female rats, 18-21 days old. The animals, including untreated normal controls, are autopsied on the second or third day at the latest and the diagnosis made from the gross changes in the reproductive organs. Serial sections are prepared from the ovaries, tubes, and uterus for confirmation of the macroscopic findings. The procedure shortens the time required for diagnosis to 36-48 hours instead of the usual 4-5 or more days.

Eight ounces of morning urine, preferably not over 8 hours old, are used in the test. Older specimens have been found satisfactory, however, and the results not vitiated by the addition of a preservative or by the reaction of the urine. A preservative such as ether tricresol (4 drops to each 100 cc. of urine) may be necessary in forwarding specimens from a distance.

Two and one-half volumes of 95% alcohol are added to the

[1] Erdheim, J., and Stume, E., *Beitr. z. Path. Anat. u. z. allg. Path.*, 1909, **46**, 1. Evans, H. M., and Long, *Anat. Rec.*, 1921, **21**, 62. Smith, P. E., and Engle, E. T., *Am. J. Anat.*, 1927, **40**, 159; *Am. J. Physiol.*, 1927, **80**, 114. Evans, H. M., and Simpson, M. E., *J. Am. Med. Assn.*, 1928, **91**, 1337. Allen, W. M., *Am. J. Physiol.*, 1930, **92**, 127, 612. Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 806; *Am. J. Physiol.*, 1929, **90**, 329; *J. Biol. Chem.*, 1930, **86**, 499.

urine and the mixture placed in the ice chest at a temperature of 2-4°C. for several hours or over night to allow the precipitate to settle out. This step may be hastened by centrifuging the mixture as soon as the precipitate has separated. The precipitate is suspended in 3-4 cc. of physiological salt solution, shaken thoroughly with an equal volume of ether, centrifuged to remove the ether and the extraction repeated 2 or 3 times. The saline solution after centrifuging and containing the specific hormone freed from the estrus-producing or ovarian hormones is now ready for injection into rats.

Interpretation of the test is based upon the positive findings of enlarged ovaries with visible corpora hemorrhagica or protruding follicles and "blood points".[2] The ovarian and tubal blood vessels and the uterus are definitely hypertrophied and congested. The gross picture varies with the stage of pregnancy. Microscopically the diagnostic criteria are the enlarged hemorrhagic follicles containing corpora lutea. The degree of luteinization may vary from slight invasion at the periphery to a complete filling of the entire structure. In the absence or presence of macroscopic changes the microscopic findings must always determine the diagnosis. A positive diagnosis rests on the finding of at least one corpus luteum.

The specific microscopic changes may be progressive or retrogressive and depend upon the advancing stages of pregnancy or upon its termination by abortion, miscarriage, or death of the fetus. It is noteworthy that detection of the death of the fetus is thus possible notwithstanding the fact that positive findings in autopsied rats may occur as late as 7 days after the death of a fetus *in utero*. The microscopic transitional picture in the corpora lutea is characterized by retrograde changes that are different from the specific luteinization, and assume an appearance approaching the negative stage. This observation as well as the other tinctorial and structural changes noted in the follicular cells are indicative of very early changes that are specific and especially valuable in circumstances that preclude diagnosis from the macroscopic findings alone.

A series of 175 consecutive cases has thus far been studied without an error in diagnosis. Subsequent histories and follow-up including post-operative findings have confirmed the diagnoses. A

[2] Zondek, B., *Arch. f. Gynaek.*, 1927, **132**, 76; *Klin. Wchnschr.*, 1928, **7**, 1404, 1929, **8**, 2229; *Naturwissensch.*, 1928, **16**, 1088; *Z. f. Geburtsch. u. Gynaek.*, 1928, **94**, 190; *Deutsch. med. Wchnschr.*, 1930, **56**, 295; *Arch. Gynaek.*, 1927, **130**, 1. Ascheim, S., and Zondek, B., *Arch. f. Gynaek.*, 1927, **132**, 179; *Klin. Wchnschr.*, 1928, **7**, 8; *Z. f. Geburtsch. u. Gynaek.*, 1928, **94**, 203; *Z. f. artzl. Fortbild.*, 1929, **26**, 5; *Zentralbl. f. Gynaek.*, 1929, **53**, 15; *Klin. Wchnschr.*, 1927, **28**; 1928, **30**, 1404.

number of patients had not yet missed a menstrual period at the time the test was made and a large group was represented by those who were in the first 4 to 6 weeks of pregnancy. The clinical material embraced all the usual problems in the differential diagnosis of pregnancy. These were represented by glandular insufficiency, functional amenorrheas, menopausal symptoms, uterine fibroid with or without pregnancy, complete or incomplete abortion, miscarriage, ectopic gestation, hysteria, dead fetus, and the like.

The test has proved 100% accurate in this study despite the rapid method and it differentiates pregnancy from conditions that simulate it. In medicolegal cases the test has been found valuable and equally important in cases demanding therapeutic abortion or in circumstances requiring prompt diagnosis or the exclusion of pregnancy.

No mortality has occurred among the rats in the course of injections.

5340

Incidence of "Normal" Persons Possessing Demonstrable Antibodies for Poliomyelitis Virus in Their Serum.*

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Recent observations by Aycock and Kramer[1] and by Shaughnessy, Harmon and Gordon[2] on the incidence of normal persons possessing poliomyelitis antibodies in their blood serum are of considerable interest not only from the standpoint of the epidemiology of this disease, but also from that of its serum therapy. The observations of Shaughnessy and his associates indicate that the titer of these antibodies in the serum of certain "normal" adults may not only equal, but appreciably exceed that of the average poliomyelitis convalescent. These important observations have prompted us to make a similar survey in this region.

The Aycock strain of the virus is being used in our studies. This strain produces poliomyelitis in rhesus monkeys with great regularity, the first symptoms of the disease appearing generally between

* These studies were supported by the Mary Hooper Somers Medical Research Fund.

[1] Aycock and Kramer, *J. Prev. Med.*, 1930, **4**, 189, 201.

[2] Shaughnessy, Harmon and Gordon, *J. Prev. Med.*, 1930, **4**, 463.

the 5th and 8th day. Pieces of cord and medulla were weighed and very finely ground in a motorized mortar[3] in the presence of sterile ground pyrex glass. Enough neutral physiological saline solution was then added to this ground material to make a 5% suspension of the virus-tissue. After light centrifugation for 10 minutes and filtration through sterile filter paper this suspension was distributed in 1 cc. quantities to a series of serological tubes containing equivalent volumes of the various sera (or serum dilutions) to be tested. In the first series of tests these serum-virus mixtures were incubated at 37°C. for 2 hours. In the second series no incubation preceded the injection of the serum-virus mixtures. The various serum-virus mixtures were injected into the frontal lobe of the brain of *M. rhesus* monkeys in doses of 1.5 cc. Monkeys which were protected against injection by a given serum dilution were again used in testing the same serum in the next higher dilution 15 to 20 days later. This procedure has been tentatively adopted by us to economize on the number of monkeys which would otherwise be required in estimating the actual antibody content of these sera. Thus far no immunizing action of such serum virus mixtures has been detected. Should this later prove to be the case, it will not be without interest. Each series was adequately controlled not only with normal monkey, but also monkey poliomyelitis convalescent serum-virus mixtures. To insure more uniform results 10 or more human sera were tested at one time.

Tests were run on the sera of a total of 32 "normal" adults, persons who to their knowledge had never suffered from recognizable poliomyelitis. Thirteen of these are included in the first series, in which the serum-virus mixtures were incubated at 37°C. for 2 hours before injection into monkeys. Of this number 9 inactivated the virus in serum dilutions of 1-2.† Only 2 of the sera in this first series were tested in the next higher dilution (1-30). Both failed to protect in this dilution. The ages of the persons tested in this series ranged from 16 to 48 years; most of them were in the early twenties. Of those which neutralized the virus, a boy and his father represent the 2 age extremes, a third member of the family (25 years of age) tested in the following series, also neutralized the virus. Nineteen sera were tested in the second series, in which the mixtures were injected without preliminary incubation. Of these 9 inactivated the virus when the serum was diluted 1-2. It is not clear whether this lower incidence is to be attributed to the lack of

[3] Schultz and Banham, *Am. J. Pub. Health*, 1930, **20**, 771.

† Virus suspension 1 part, undiluted serum 1 part.

incubation, or to a lower incidence of neutralizing sera in this series. The ages of the persons in this series ranged from 20 to 52 years, the majority being under 25 years. Of the 9 which inactivated the virus, only 5 have thus far been retested in the next higher serum dilution (1-30). Two of these failed to neutralize the virus in this serum dilution and 3 not only protected in this dilution, but also in a dilution of 1-60. Tests have not yet been carried out to determine whether these monkeys have become refractory.

These studies are not sufficiently advanced to permit a generalization as to the actual incidence of persons possessing poliomyelitis immune substances in their blood nor to throw any further light on the concentration of antibodies represented in these sera. Nevertheless, the results tend to confirm the observations of previous investigators. There is little doubt that were tests carried out in the classical manner inaugurated by earlier investigators, such as Flexner, in which the ratio of serum to virus is much greater, a larger incidence of poliomyelitis immune persons could be detected in the normal adult population. We plan to test this hypothesis. As to the incidence of persons whose serum will titer as high, or higher than the average human convalescent serum, much more work is necessary to reveal this relationship. There is sufficient evidence to suggest that the incidence may be of fair magnitude. That not all human convalescent serum is capable of inactivating the virus even when favorable relationships obtain is well recognized. That the antibody level of the average convalescent serum may indeed be relatively low is suggested by the recent work of Shaughnessy and his associates. Of 4 human poliomyelitis convalescent sera, run as auxiliaries in our series, 2 neutralized in a serum dilution of 1-2, while 2 did not. The 2 which protected in this dilution, failed to do so in a serum dilution of 1-30. How the viricidal properties have been acquired by persons who have no knowledge of having had the disease is not entirely clear. Aycock and Kramer[4] have adduced evidence that the immunity is a gradual acquisition, increasing with the age of the individual. They found that new born children of mothers possessing viricidal antibodies in their serum, present the same properties in their blood for a time. Thereafter the sera of younger children apparently quite uniformly fail to show this property. The natural inference is that the immune principle is acquired as a result of subclinical infections with poliomyelitis virus, though it is possible that it may not rest altogether on this basis.

[4] Aycock and Kramer, *J. Exp. Med.*, 1930, **52**, 457.

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Antipoliomyelitis Serum Production in the Horse.*

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In a previous note[1] we reported our inability to demonstrate an antibody response in poliomyelitis refractory animals in any way comparable to that realized in monkeys. Although antisera produced in the guinea pig, rabbit, dog, sheep and goat in some instances inactivated an equal volume of a 5% suspension of virus tissue (cord and medulla) when these sera were employed in an undiluted form, previous dilution of the serum to 1-2 (after addition of virus suspension, 1-4) failed to render such a virus suspension non-infectious for monkeys. Essentially the same results were obtained with the serum of a horse, which at the time of the previous report (June, 1930) had been given 9 intravenous injections of 50 cc. (one injection 20 cc.) each of a 10% suspension of virus material (cord and medulla) over a period of 45 days. Since the horse represented a much later addition to our series of poliomyelitis refractory animals, virus injections have been continued to make the period of "immunization" more comparable to those of the sheep and goat, which received virus injections over a period of about a year. The results with the horse have, however, proven far more gratifying than in the case of the other poliomyelitis refractory animals. Titrations carried out recently on serum procured from the horse 8 months after the "immunization" was begun indicate that the serum is now capable of inactivating an equal volume of a 5% virus suspension in a final serum dilution of 1-60, which according to the recent observation of Shaughnessy, Harmon and Gordon[2] may equal, if not exceed the antibody titer of the average human poliomyelitis convalescent serum. The antibody titer is not as high as that of some of the hyperimmunized poliomyelitis convalescent monkey sera we have titrated (1-128), but we anticipate that with further immunization of the horse the titer may rise to a higher level. It should be added that the *normal* serum of this animal neutralizes equivalent amounts of the virus in a final serum dilution of 1-2, but not in a serum dilution of 1-4.

* These studies were supported by the Mary Hooper Somers Medical Research Fund.

[1] Schultz and Gebhardt, PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 31.

[2] Shaughnessy, Harmon and Gordon, *J. Prev. Med.*, 1930, **4**, 459.

The results, therefore, indicate that different species of refractory animals differ widely in their responsiveness as antibody producers against this particular virus. Different members of the same species may also vary widely in this respect. Fairbrother and Morgan,[3] for example, have noted that the responsiveness of two horses "immunized" with poliomyelitis virus differed greatly, one producing a good viricidal serum, the other not responding at all.

5342

Occurrence of Infectious Myxomatosis in Southern California.

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During the summer of 1930 twelve reports from rabbitries in the regions of Santa Barbara, Ventura, and San Diego were made of a disease presenting the following symptoms. The rabbits were acutely ill and exhibited, as a rule, an oedematous condition in the regions of the nose and lips, the external genitalia and a conjunctivitis. A purulent discharge occurred from the eyes and nose. The ears also became greatly thickened and drooped as a rule. Animals that lived longer than a week or 10 days after the appearance of symptoms often developed nodules around the nose, eyes, or on the ears. Upon autopsy, the lymph nodes and spleen were found usually to be enlarged. The nodules and oedematous areas were found to contain a gelatinous material.

The disease was transmitted with facility by rubbing the discharge from the eyes and nose, or the extract from tissues, into the skin, or by injecting the same. Comparison of the above findings with reports of Hobbs,[1] Rivers[2] and others who have studied infectious myxomatosis produced by the South American virus shows striking similarities, the chief difference being that the disease encountered in California is not transmitted with as great ease by contact among experimental animals as is the South American disease. First attempts to transmit the disease by filtrates passed through

[3] Fairbrother and Morgan, *Brit. J. Exp. Path.*, 1930, **9**, 298.

[1] Hobbs, J. R., *Am. J. Hyg.*, 1928, **8**, 800.

[2] Rivers, T. M., *J. Exp. Med.*, 1930, **51**, 965.

medium Berkefeld filters were negative, but later attempts with coarser filters were positive.

Dr. Rivers has exchanged histopathological slides and virus and after studying our slides states: "From your sections there is good reason to suppose that the disease you are working with is the so-called infectious myxomatosis of rabbits."

Certain comparative experiments of the California strain with the South American strain provided by Dr. Rivers are recorded in Table I.

TABLE I.

	California Strain				South American Strain			
Aver. incubation period	7 days				5½ days			
Aver. days living after appearance of symptoms	5 "				3½ "			
Contact Experiments	+ 4		— 6		+ 10		— 0	
Filtration Experiments	Berkefeld Coarse V		Berkefeld Medium N		Berkefeld Coarse V		Berkefeld Medium N	
	+ 5	— 6	+ 1	— 12	+ 4	— 0	+ 0	— 4

From this table it is seen that the average incubation period of the South American strain is 5½ days as compared with 7 days for the California strain. The average length of life after the appearance of symptoms is 3½ days in the South American strain and 5 days in the California strain. These data combined with the fact that the South American strain is transmitted with greater ease by contact than the California strain indicate that at present the California strain of virus exhibits a lower degree of virulence than the South American strain provided by Dr. Rivers. It should be remembered, however, that this South American strain has been repeatedly passed through experimental animals since being brought to the United States and its virulence may thereby have been increased. The filtration experiments indicate that the California strain of virus possesses approximately the same filter passing power as the South American strain.

All animals encountered to date in this study, whether naturally or experimentally infected, have died.

Our findings indicate that infectious myxomatosis has been encountered in a natural outbreak in Southern California, which is its first natural appearance outside of South America.

5343

Kinetics of Anaerobic Recovery in Muscle Contraction.

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In an earlier paper[1] the authors called attention to the fact that the lactic acid liberated per contraction in striated muscle made to contract isometrically is dependent upon the interval between contractions, and presented a curve relating lactic acid produced per contraction to the contraction frequency. The location of at least 2 of the points on the earlier curve, at frequencies of 12 per second and 4 per second, was only approximate, since the number of experiments at those frequencies was small. We have since conducted a larger number of experiments, approximately 50 each at frequencies of 12 per sec., 6 per sec., 4 per sec., 2 per sec., 1 per sec., and 1 per 2 seconds all at 22°C. We believe the number to be sufficient to indicate with reasonable accuracy the course of the time interval-lactic acid curve. The standard deviation is in each case about 0.02 mg. per 100 gm. of muscle. Stimulation was through the nerve by

FIG. 1.

[1] Visscher, M. B., and Smith, P. W., *Am. J. Physiol.*, 1930, **95**, 121.

means of maximal break shocks from a Harvard inductorium, through a mechanical circuit breaker and make-shock eliminator.

In the earlier paper it was shown that at infinite intervals between contractions, (when the interval becomes greater than 2 seconds at 22°) the lactic acid produced per stimulation becomes constant. Evidence was presented to show that at intervals shorter in duration, removal is not complete. If we may assume the concentration of lactic acid reached when intervals are infinite to be a concentration maximum, and that in each successive contraction the concentration of lactic acid is built up to that maximum, then the amount of new lactic acid which appears in each contraction is an index to the amount removed in the preceding interval. From the data given in Fig. 1 relating lactic acid per 100 gm. of muscle per contraction to the time interval between contractions we have computed the percentage recovery in a given time interval, as presented in Table 1.

TABLE I.

Percentage Recovery in Terms of Lactic Acid Removed in Intervals Between Contractions.

Mgm. Lactic Acid per 100 gm. Muscle per Contraction	Time in Seconds	% Recovery
0.43	0.084	30.2
0.85	0.167	59.8
0.97	0.250	68.3
1.15	0.500	81.0
1.35	1.000	95.0
1.42	2.000	100.0

With a necessary correction to allow for the fact that recovery actually begins after the excitation disappears rather than at the instant of stimulation, the results above can be used to show that the reaction proceeds with a velocity characteristic of a first order reaction. Such a result might have been predicted from a consideration of the fact that during the course of a single recovery the free lactic acid concentration changes from 100% to a very small fraction of its contraction level, whereas during the same single recovery period the concentration of the substance combining with it in its removal, changes only to a small extent. Consequently the concentration of one reactant governs the velocity and the reaction appears as one of the first order over any single recovery period.

5344

"Castration Cells" in Anterior Hypophysis of Spayed Rat Following Prolonged Administration of Estrin.*

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The occurrence of a characteristic cell in the anterior lobe of the hypophysis of spayed animals has been recognized since the pioneer work of Fichera in 1904, and 2 important biological phenomena have recently been demonstrated accompanying this histological change. It has been found that implants of the anterior lobe of castrated rats are much more potent in inducing precocious maturity than those from normal animals,[1, 2] possibly due to a storage of the hormone by the castration cells.[2] It has also been found that the blood[3] and urine[4] of women after bilateral oophorectomy contains large amounts of an ovary-stimulating substance as determined by the Ascheim-Zondek test.

Since it has also been suggested that estrin (ovarian follicular hormone) has the power of inhibiting the ovary-stimulating action of the anterior hypophysis (Siegmund[5]; Mahnert[6]; Dahlberg and Akesson[7]; Meyer, Leonard, Hisaw and Martin[8]), it seemed of importance to determine if the constant administration of large amounts of estrin to spayed rats would influence the formation of the castration cells.

Two series of animals have been studied. (1) Seven adult female rats were spayed, and sacrificed 90 days later. During this time 4 of them were given subcutaneous injections of 5 rat units of estrin (Amniotin-Squibb) every third or fourth day, so that 3 received a total of 135 and one of 180 units. (2) Five adult fe-

* The second experiment of this investigation was made possible by a donation of Amniotin by Messrs. E. R. Squibb & Sons.

1 Engle, E. T., *Am. J. Physiol.*, 1929, **88**, 101.

2 Evans, H. M., and Simpson, M. E., *Am. J. Physiol.*, 1929, **89**, 371.

3 Fluhmann, C. F., *J. Am. Med. Assn.*, 1929, **93**, 672; *Am. J. Obstet. and Gynec.*, 1930, **20**, 1.

4 Zondek, B., *Klin. Wchnschr.*, 1930, **9**, 393.

5 Siegmund, H., *Zentrlbl. f. Gynaek.*, 1928, **52**, 1189.

6 Mahnert, A., *Zentrlbl. f. Gynaek.*, 1928, **52**, 1754.

7 Dahlberg, G., and Akesson, S., *Acta Obstet. Scand.*, 1930, **10**, 63.

8 Meyer, R. K., Leonard, S. L., Hisaw, F. L., and Martin, S. J., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 702.

male rats, littermates, 166 days old, were spayed. Three were given subcutaneous injections of 5 to 10 rat units of estrin (Amniotin-Squibb) every second day until each had received a total of 215, 220, and 225 rat units respectively. They were sacrificed 77 days after castration.

In each instance serial sections of the anterior lobe were studied, and it was found that there was no demonstrable difference in the character or number of "castration cells" between the control and experimental animals. It would seem, therefore, that the absence of estrin stimulation in castrates is not *per se* the factor concerned in the formation of castration cells.

New York Section.

New York Academy of Medicine, December 17, 1930.

5345

Studies on the Etiology of Rheumatoid Arthritis. I. Bacteriological Investigations on Blood, Synovial Fluid and Subcutaneous Nodules in Rheumatoid Arthritis.*

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The clinical evidence pointing to an infective origin of rheumatoid arthritis has led numerous investigators to seek a bacterial agent to which etiological significance could be ascribed. A wide variety of such agents has been reported, but lack of uniformity in the results obtained has confused rather than clarified the issue. Cecil, Nicholls and Stainsby,[1] by the use of a special technique, claim to have demonstrated the presence of streptococci in the blood-stream in 61.5% of a series of 78 cases examined. Because of the unusual nature of this report and the importance of such a finding the present investigation was undertaken.

The greatest care was exercised to follow their technique. The type of patient selected in all cases presented the characteristic clinical syndrome of rheumatoid arthritis. Cases of osteo-(hypertrophic, degenerative) arthritis were not included.

One hundred and five separate blood cultures were done on 80 patients. In the majority of instances the specimens of blood obtained at each vene puncture were divided into 2 portions so that in all 204 samples of blood were cultured. Eighteen selected patients were cultured on 2 occasions, 3 on 3 occasions and one on 6 occasions.

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* The term "Rheumatoid Arthritis" is synonymous with the terms "Chronic Infectious" and "Atrophic Arthritis".

† The Arthritis Clinic of the Presbyterian Hospital is supported by the Faulkner Memorial Fund.

[1] Cecil, R. L., Nicholls, E. E., and Stainsby, W. J., *Arch. Int. Med.*, 1929, **43**, 571.

As a control 31 samples of blood were obtained from 16 normal individuals and subjected to similar methods of culture. Sixteen tubes of sterile, autoclaved agar were subjected to the same manipulations and cultured by the same technique.

The results may be summarized as follows: (1) In spite of the greatest care to conduct all manipulations under sterile precautions the technique was so involved as to call into serious question the significance of all bacterial growth encountered. (2) Blood cultures on patients suffering from rheumatoid arthritis failed to yield results which could be considered of etiological significance. (3) No essential difference was found in the variety and character of the bacteria encountered during the culture of specimens of blood from patients with rheumatoid arthritis and of the control material. (4) On 2 occasions colonies of typical *Streptococcus viridans* appeared during the culture of specimens of sterile agar subjected to similar manipulations.

Joint Cultures.—Twenty-three samples of synovial fluid, obtained from 19 patients suffering from rheumatoid arthritis, were cultured both aerobically and anaerobically in a variety of media which included the following: blood broth, hormone broth, meat broth, dextrose ascitic broth and dextrose ascitic agar. All the specimens were incubated for at least 30 days and carefully examined for the presence of bacterial growth at 4 to 5 day intervals during that time. The aerobic and anaerobic cultures of these 23 samples of synovial fluid failed to yield organisms which could be considered of etiological significance.

Cultures of Subcutaneous Nodules.—It has been pointed out[2] that the subcutaneous nodules frequently seen in rheumatoid arthritis constitute a classical lesion of this disease. Careful bacteriological studies were carried out on a series of 16 subcutaneous nodules obtained from 11 patients suffering from rheumatoid arthritis. Aerobic and anaerobic cultures of these nodules in a wide variety of media failed to yield organisms which could be considered of etiological significance.

[2] Dawson, M. H., and Boots, R. H., *J. Lab. and Clin. Med.*, 1930, **15**, 1065; *J. Am. Med. Assn.*, 1930, **95**, 1894.

5346

Studies on the Etiology of Rheumatoid Arthritis. II. Agglutination Reactions with Hemolytic Streptococci in Rheumatoid Arthritis.

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The present authors reported[1] bacteriological investigations on blood, synovial fluid and subcutaneous nodules in rheumatoid arthritis, which entirely failed to confirm the results of Cecil, Nicholls and Stainsby. Cecil, Nicholls and Stainsby[2] further stated that the sera of patients suffering from rheumatoid arthritis possessed the property of agglutinating their *"typical strains"* to a remarkably high titre.

Through the courtesy of Dr. Cecil several *"typical strains"* were made available. Specimens of serum were obtained from a large number of patients suffering from rheumatoid arthritis and agglutination tests were done using these strains as agglutinogens. For control purposes a large number of other organisms, obtained from a variety of sources, was similarly employed against the sera of patients with rheumatoid arthritis. The study was further controlled by utilizing a large number of specimens of serum obtained from patients suffering from both related and unrelated diseases.

In addition to the "typical strains" of Cecil, Nicholls, and Stainsby, which, in the authors' experience, showed varying degrees of hemolytic properties, cultures of the following organisms were employed in the agglutination tests: (1) *Streptococcus hemolyticus,* 4 strains; these included strains obtained from scarlet fever, erysipelas and from the throat of a patient with rheumatic fever; (2) *Streptococcus viridans,* 7 strains; (3) *Streptococcus anhemolyticus,* 12 strains; (4) green diplococci (exact nature undetermined) 3 strains; (5) *Staphylococci,* 5 strains.

Specimens of sera obtained from 66 patients suffering from typical rheumatoid arthritis were employed during the course of this study. Control sera were obtained from 50 cases of both related and unrelated diseases. These diseases included the following:

[1] Dawson, M. H., Olmstead, Miriam, and Boots, R. H., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 419.

[2] Cecil, R. L., Nicholls, E. E., and Stainsby, W. J., Scientific Proc., 30th Annual Meeting Am. Assn. Path. and Bact., New York, April, 1930; Rep. of 45th Annual Meeting, Assn. Am. Physicians, Atlantic City, May, 1930; *Am. J. Med. Sci.,* 1931, **181**, 12.

osteo-arthritis, 18 cases; gonococcal arthritis, 4 cases; spondylitis, 8 cases; intermittent hydrarthrosis, 1 case; subacute rheumatic fever, 6 cases; non-articular rheumatism, 4 cases; other diseases—chronic nephritis, thromboangiitis obliterans, gastric ulcer, lung abscess, pulmonary tuberculosis, pneumonia, sacro-iliac strain, neurosis, tuberculous peritonitis—9 cases.

The results of over 1000 agglutination tests, using 37 different cultures as agglutinogens on 66 cases of rheumatoid arthritis and 50 control cases have led to the following conclusions: (1) In the great majority of cases sera of patients with rheumatoid arthritis possess the property of agglutinating hemolytic streptococci to an extraordinarily high titre. (2) Strains of *Streptococcus hemolyticus* obtained from scarlet fever, erysipelas, and from the throat of a patient with rheumatic fever were agglutinated by these sera to as high a titre as were the "typical strains" of Cecil, Nicholls and Stainsby. (3) Absorption tests carried out with *Streptococcus hemolyticus* from scarlet fever, erysipelas and the "typical strains" of Cecil, Nicholls and Stainsby failed to show any evidence of specificity of the agglutination reaction for the various strains of *Streptococcus hemolyticus* examined. (4) Of the 50 control sera only 2 showed evidence of agglutinins for the strains of *Streptococcus hemolyticus* employed. In these 2 instances the agglutination was of a very low titre and of doubtful significance. (5) Of 31 strains of other organisms used none was agglutinated by the sera of patients with rheumatoid arthritis to any significant titre.

The present study supports the following hypothesis: Rheumatoid arthritis, in the majority of instances, results from infection with *Streptococcus hemolyticus*. The evidence so far accumulated indicates that no specific strain of *Streptococcus hemolyticus* can be considered as the sole etiological agent but this phase of the problem is being further investigated. No evidence has been obtained that the organisms gain access to the circulation or the joint tissues. The suggestion is therefore advanced that the majority of cases of this disease represent the response of the affected tissues to products of *Streptococcus hemolyticus* absorbed from a distant focus.

5347

The Effect of Various Carbohydrates on Production of Diphtheria Toxin with Special Reference to its Flocculating Power.

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From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

Park and Williams,[1] following the work of Spronck,[2] reported that small amounts of glucose added to the broth aided in production of potent toxic filtrates from *Corynebacterium diphtheriae*. They warned at the same time that an excess of glucose sufficient to cause too great a degree of acidity would inhibit the development of the toxin. T. Smith[3] also found that small quantities of dextrose were favorable to toxin production, provided the muscle sugar had been removed from the broth by fermentation. Recently Locke and Main[4] and Ramon[5] have again called attention to the use of glucose in the production of high-titred diphtheria toxin.

We have studied the effect of a number of carbohydrates, fermentable by *C. diphtheriae*, on the production of toxic filtrates of high Lf* unitage. We are reporting here only those experiments in which glucose and maltose have been used to enrich the culture medium.

The culture filtrates (Park-Williams No. 8 strain of *C. diphtheriae*) were obtained after incubation from 48 hours to 5 days at 37.5°C., no preservative being added. The pellicles were grown in every case in one litre flasks containing 250-300 cc. of broth as used by Povitsky.[7] The initial inoculum consisted of one large loopful of an actively growing pellicle, the seed culture having been maintained previously by rapid transfer, twice daily. Apparently little harm was done to the pellicles by manipulation of the flasks in adding the sugars and adjusting the pH of the culture fluid. Toxins

[1] Park, W. H., and Williams, A. W., *J. Exp. Med.*, 1896, **1**, 164.

[2] Spronck, C. H. C., *Ann. de l'Inst. Past.*, 1895, **9**, 758.

[3] Smith, T., *J. Exp. Med.*, 1899, **4**, 373.

[4] Locke, A., and Main, E. R., *J. Inf. Dis.*, 1928, **43**, 41.

[5] Ramon, G., *Compt. Rend. Soc. de Biol.*, 1929, **101**, 718.

* The term is used in accordance with Glenny and Wallace's definition indicating "that amount of toxin (corresponding to one unit of a certain antitoxin) in that mixture which flocculates most rapidly when a series of mixtures of that toxin and antitoxin are set up in varying proportions and observed under constant conditions."[6]

[6] Topley and Wilson, *Principles of Bacteriology and Immunity*, **2**, 865.

[7] Povitsky, O., *J. Immunol.*, 1929, **16**, 421.

were obtained within 48 hours from cultures grown in this basic medium which had 6.5 Lf units per cc. and approximately 500 M.L.D. per cc.

The two sugars have been utilized as follows: (1) Fractional amounts of either glucose or maltose were added to the growing culture from one to 3 times daily for 48 to 72 hours, each addition of sugar being preceded by titration and adjustment of the culture fluid to pH 8.0. Two control flasks with the same sugar concentrations, but which remained unadjusted, were carried along at the same time. (2) A definite amount of each sugar was added to the broth before planting the pellicle with daily titration of the culture medium. (3) In another experiment a given amount of the 2 sugars in combination was added to the broth before planting the pellicle, with daily adjustment of the culture fluid to pH 8.0; control flasks of similar composition but unadjusted pH were run at the same time.

The Ramon flocculation test was carried out in the routine manner[8] on the various filtrates. In those cases where there were repeated titrations in a single day, multiple flasks were run. One sample of fresh antitoxic horse serum† (350 Ehrlich units per cc.) was employed throughout the entire series.

Only approximate M.L.D. determinations were made on the majority of the filtrates. In those instances, however, where the M.L.D. was determined, it was not uncommon to obtain frequently filtrates which would kill guinea pigs of 250-300 gm. weight within 5 days after subcutaneous injection of 1/1000 cc., 1/1400 cc., or even as little as 1/1900 cc.

Our observations may be briefly summarized. Filtrates of high Lf titre (15 units per cc.) were obtained within 48 to 72 hours by semi-daily additions of glucose alone (0.15%), provided there was an adjustment of the hydrogen ion concentration after each addition of the sugar to pH 8.0. Under such conditions the pH at no time fell below 6.7, while without adjustment values as low as 5.6 were attained. The pronounced deleterious effect of the acid reaction on the toxin is demonstrated by the fact that unadjusted filtrates yielded toxins with an Lf titre of only half the value obtained in the more alkaline medium. Very small amounts of maltose alone (0.075%), added 3 times daily, to the growing culture, were found to yield within 48 to 72 hours toxins of considerable strength (8 Lf

[8] Hazen, E. L., *J. Immunol.*, 1930, **19**, 393.

† Kindly supplied by Dr. J. F. Anderson of the Squibb & Sons Biological Laboratories.

units per cc.) although toxin production could be conspicuously enhanced by the addition of larger amounts of this sugar and prolongation of the incubation period to 5 days. In no instance was it necessary to adjust the culture fluid since the pH never fell below 6.8. Toxins of unusually high Lf value were obtained within from 3 to 5 days in a medium which contained both sugars (0.15% glucose and 0.3% maltose), added in combination before inoculation. In experiments carried on in the summer months, filtrates titrating as high as 26.3 Lf units per cc. were harvested with this particular method within 5 days. At other times the filtrates always contained 17 to 20 units per cc. Such filtrates moreover flocculated within 20 minutes, indicating high antigenicity (Schmidt[9]). Both adjusted and unadjusted culture fluids gave approximately the same values.

In conclusion, it may be stated that while either glucose or maltose definitely enhanced toxin production, filtrates of highest potency are obtained in a medium containing both sugars (0.15% glucose 0.3% maltose = 0.45% total carbohydrates). It would seem that the latter method permits of a more rapid and a more abundant toxin production than is commonly known.‡

5348

Passive Local Sensitization in Atopic Individuals.

MATTHEW WALZER AND KATHERINE BOWMAN.
(Introduced by M. J. Shear.)

From the Department of Bacteriology and Immunology, Cornell University, Medical College, New York Hospital, and the Jewish Hospital, Brooklyn.

The technic for studying the absorption of unaltered proteins in humans has been described.[1] A cutaneous site is passively and locally sensitized with a small amount of serum taken from an atopic patient who is extremely sensitive to the protein to be tested. On the following day, the specific protein is fed to the subject on an empty stomach. Within a few minutes to a few hours, a wheal forms at the sensitized site demonstrating roughly the rapidity and,

[9] Schmidt, S., *Ann. de l'Inst. Past.*, 1930, **45**, 357.

‡ We wish to express our thanks to Mr. Soo-Hoo of the Department of Practice of Medicine, Physicians and Surgeons, Columbia University, for his kind assistance on this work.

[1] Walzer, M., *J. Immunol.*, 1927, **14**, 159.

to a certain degree, the amount of unaltered protein absorption in that subject. The results of studies with various proteins and in different types of subjects have already been presented.[2, 3]

The technic may, however, fail completely or may show diminished reactions in atopic subjects. One of the factors which accounts for this is that atopic individuals do not accept passive local sensitization as well as normals. The evidence is submitted herewith.

The ability of atopics to accept passive local sensitization was determined by titrating on their skins the sensitizing power of certain atopic sera of known strength.

The titrations of these sera were performed according to the method of Coca and Grove[4]. Atopic and normal subjects were sensitized with the serum in a range of dilutions determined to be suitable for that serum by previous titration on normal subjects. Seven days after sensitization the sites were tested with a suitable dilution of the atopen for which the serum in question contained reagins. Control tests on normal skin sites were introduced at the same time. Readings were made according to the method used in the indirect method of testing[5]; *i. e.,* any definite excess of reaction, either in wheal or in erythema, on the sensitized site over that manifested on the control site was considered a positive transfer.

The results of 5 titrations of atopic sera on normal and atopic individuals are presented in the table. Only the highest dilution which succeeded in sensitizing the skin of each subject is recorded in the table. In every serum, the same sensitizing dilutions were tried on both the normal and the atopic subjects.

With each of the sera tested it can be seen that the atopic patients did not as a group accept passive local sensitization with the same regularity or to the same degree as the normals. This was particularly true of sera containing reagins for *Ascaris lumbricoides.* Some of the patients who failed to accept passive local sensitization with the latter sera could be sensitized with a rabbit epithelium serum. It would seem therefore that the nature of the sensitizing serum is also a factor to be reckoned with in passive local sensitization. Regardless of the sensitizing serum it may be definitely stated that atopic individuals do not accept passive local sensitiza-

[2] Brunner, M., and Walzer, M., *Arch. Int. Med.,* 1928, **42**, 172.

[3] Sussman, H., Davidson, A., and Walzer, M., *Arch. Int. Med.,* 1928, **42**, 409.

[4] Coca, A. F., and Grove, E. F., *J. Immunol.,* 1925, **10**, 445.

[5] Walzer, M., *J. Allergy,* 1930, **1**, 231.

TABLE I.

Serum	Dilutions of Sensitizing Serum		Failed to accept passive local sensitization in stated dilutions.	Accepted Passive Local Sensitization						
	Subjects	Number Tested	%	1:4 %	1:10 %	1:40 %	1:80 %	1:160 %	1:320 %	1:640 %
Rabbit Epithelium Serum "K"	Atopics	15			6.6	33.3		60		
	Normals	8					12.5	87.5		
Rabbit Epithelium Serum "K" (Aged in icebox 3 months.)	Atopics	9				33.3		66.6		
	Normals	10						100		
Ascaris Lumbricoides Serum "S"	Atopics	23	30.4*				8.7	17.4	30.4	13
	Normals	13	7.7					30.7	23	38.4
Ascaris Lumbricoides Serum "G"	Atopics	10		100						
	Normals	9		11.1		88.8				
Timothy Pollen Serum "C"	Atopics	22			4.5	9	50	36.3		
	Normals	7					57	43		

* Two of these 7 subjects were sensitized with the undiluted "S" serum and failed to demonstrate a transfer of reagins. A third accepted passive local sensitization in a dilution of 1:10. The remaining 4 atopics and the normal were not tested with a dilution of the serum below 1:40.

tion as well as normals. This fact must be taken into consideration in the interpretation of the results of the absorption phenomenon in atopic patients.

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Effect of Extract of Adrenal Cortex upon Thymic Weight and Resistance to Bacterial Intoxication in Chronic Adrenal Insufficiency.

W. J. MERLE SCOTT AND W. L. BRADFORD.

From the Departments of Surgery and Pediatrics, the University of Rochester School of Medicine and Dentistry.

Extirpation of both adrenal glands is rapidly fatal in most laboratory animals. However this operation seems to have little effect for several weeks upon the physical condition of the majority of rats submitted to it. But such apparently healthy survivors manifest at least 2 important differences from normal rats, an increased susceptibility to bacterial intoxication,[1] and hypertrophy of the thymus.[2] In each instance the variation has been correlated with a functional deficiency of the adrenal cortex rather than of the medulla.

It is our purpose to report the effect of an extract of the adrenal cortex upon this chronic type of adrenal insufficiency, using as criteria the resistance of the animals to bacterial intoxication and the size of the thymus.

Fifty-eight rats from 5 litters born on the same day were used. These were divided into 3 groups and sampled for the different groups according to the method adopted by Dr. Luce Clausen,[3] insuring equal distribution of weight and equal representation of the different litters in each group. At the beginning of the experiment the mean actual weights of the 3 groups were as follows:

Group 1—97 gm. ±1.7.
" 2—95 " ±1.4.
" 3—95 " ±2.2.

Each of the first 2 groups contained 19 members. Group 3 was used for control determinations of body weight and thymus weight and was subdivided into 2 similar parts of 10 members each, one of which (A) received the injection of typhoid vaccine and the other (B) did not. All members of Groups 1 and 2 were doubly adrenalectomized on the same day and were injected subcutaneously with ½ cc. of fluid twice daily for 2 weeks after operation. Group 2 received an extract of the adrenal cortex made and kindly supplied to us by Drs. W. W. Swingle and J. J. Pfiffner,[4] while in Group 1

[1] Scott, W. J. M., *J. Exp. Med.*, 1924, **39**, 457.
[2] Jaffe, H. L., *J. Exp. Med.*, 1924, **40**, 325, 619, 753.
[3] Clausen, E. Luce, *J. Nutrition*, 1929, **2**, 125.
[4] Swingle, W. W., and Pfiffner, J. J., *Science*, 1930, **71**, 321.

the same amount of Ringer's solution was used. Two weeks after operation all surviving members of Groups 1, 2 and 3 A were injected intraperitoneally with 1½ cc. of standard typhoid vaccine. (One billion killed typhoid bacilli per cc.; no preservative.) This dose has previously been shown to be usually fatal to bilaterally adrenalectomized rats 2 weeks after operation (Jaffe[5]). All surviving rats were sacrificed 48 hours after the injection of the bacterial vaccine. Four of Group 1 (saline injected) and 3 of Group 2 (extract injected) died in the first week. All of these had profound infections of the respiratory tract, (pneumonia, etc.) except one of the former that apparently died of uncomplicated adrenal insufficiency. In the second week, 10 rats of Group 1 died. All except one of these fatalities occurred one day when the temperature of the animal room by accident fell several degrees over night. Only one rat in Group 2 died in the second week although this group was exposed to the same unfavorable low temperature that proved so disastrous in Group 1. This animal escaped from his cage on to the floor of the room where the temperature was several degrees cooler and remained there over night. He was in a moribund condition when found in the morning.

The gain in weight in the 3 groups was as follows:

		%			%
For the first week	Group	1— 1	For the second week	Group	1— 7
	"	2— 4		"	2—16
	"	3—17		"	3—20

Fifteen of the 19 rats in Group 2, and 5 of the 19 rats in Group 1 were surviving in good condition 2 weeks after operation and were injected with 1½ cc. of the typhoid vaccine at that time. All of the animals in Group 1, and 12 of the 15 animals in Group 2 succumbed to the bacterial intoxication within 36 hours. Three animals in Group 2 survived the injection and had recovered a normal appearance. Forty-eight hours after the injection of bacteria they were sacrificed.

At autopsy the thymus in all animals was carefully dissected out and weighed. In Table I the mean weight of the thymus with the probable error of the mean is given for each group. In the last

TABLE I.

Group	Mean Weight of Thymus, mg.	T/BW
I	392±.0265	3.9±.0001
II	389±.0183	3.2±.0001
IIIa	250±.0139	2.1±.0001
IIIb	294±.0124	2.1±.0001

[5] Jaffe, H. L., *Am. J. Path.*, 1926, **2**, 421.

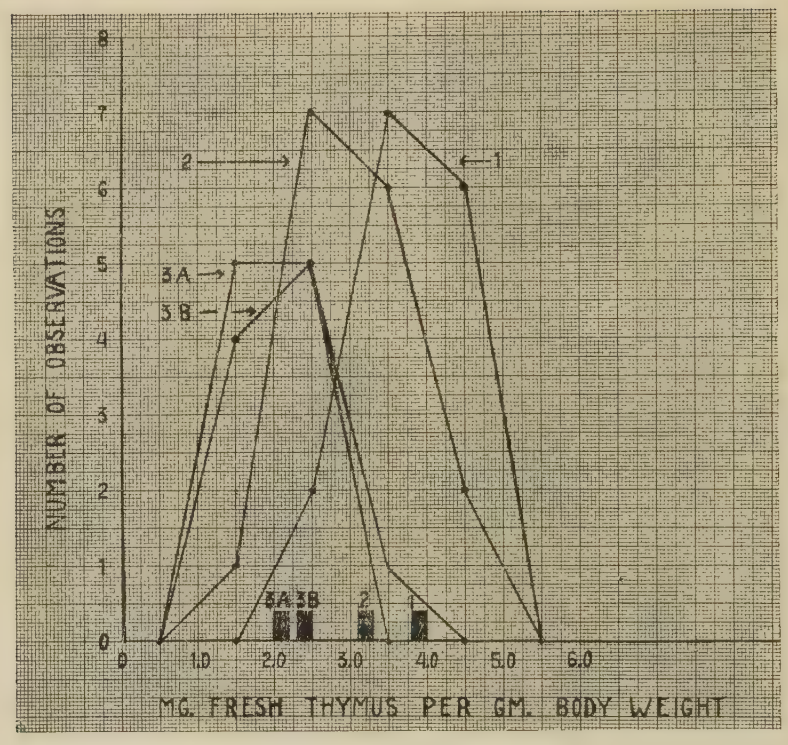

FIG. 1.

column this has been expressed in terms of mg. of fresh thymus per gm. of body weight. In order to show graphically all the observations in the 4 groups, Fig. 1 gives the data expressed in the form of frequency polygons. It is evident from this data that the size of the thymus per unit of body weight is greatest in Group 1 (saline injected), intermediate in Group 2 (cortical extract injected), and least in Group 3 (control). The difference between sub-groups IIIa (control receiving typhoid vaccine) and IIIb (control receiving nothing) is too slight to be of significance.

Two rats were found at autopsy to possess gross fragments of adrenal tissue and consequently were not classified in the above groups. One had received cortical extract injections and the other saline. They had both survived in good condition the injection of 1½ cc. of typhoid vaccine. They constitute another type of control, as they possessed functioning adrenal cortical cells. The weights of the thymus were 318 and 193 mgm. with body weights of 122 and 116 gm. respectively or 2.6 and 1.7 mgm. per gm. of body weight. These correspond closely to the greatest frequency of thymic weights in the control groups IIIa and IIIb.

The cortical extract used seemed definitely to produce an effect upon the chronic form of adrenal insufficiency in rats. The mortality occurring about 3 or 4 days after operation, due chiefly to respiratory infection, did not appear to be much affected. However, the mortality of the second week, augmented in this case by accidental chilling was largely prevented by the extract. Its beneficial effect was also shown by the resumption of practically a normal growth curve in the second week, while the saline injected control animals gained less than half as much in this period. The weight of the thymus per gm. of body weight in the extract injected group was intermediate between that of the saline injected group and that of the controls. The extract seemed also to decrease slightly the susceptibility of the adrenalectomized rats to killed typhoid bacilli.

We are of the opinion that a more complete protection in this chronic form of adrenal insufficiency can be demonstrated by pushing the administration of extract just before and during the bacterial intoxication. Such an attempt is now in progress.

We want to thank Drs. Swingle and Pfiffner for furnishing us with the extract of the adrenal cortex used.

5350

Relation of Various Substances Used in the Artificial Feeding Mixtures of Infants to Nutritional Anemia.

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The investigation made for the purpose of determining the influence on the hemapoietic system of various substances commonly used in infant feeding included a study of both rats and infants.

In the work with rats, substances recommended for infant feeding: carbohydrate milk modifiers, orange juice, tomato juice, autolyzed yeast, liver, egg yolk, Vitamin B containing extracts, and various iron and copper additions, were tested. These were fed in conjunction with milk in proportions comparable to those used in infant feeding. Unmodified milk diets including both boiled and pasteurized were included, also, for comparison. Fifty-seven animals were fed, 2 to 3 on each diet, for 6 to 18 weeks. Hemoglobin determinations (Newcomer method) were made at the beginning of experimental diet and after 7 weeks of the diet. Records of

food ingestion and body weights were kept to see what relation these have to hemoglobin formation.

The results of the investigation indicated that carbohydrate milk modifiers used in infant feeding differ in their ability to bring about hemoglobin formation. In rats lactose and sucrose seemingly have no influence since hemoglobin values for those receiving milk diets with these additions were no higher than for those receiving milk alone; Dextri-Maltose, corn syrup, Vitavose, or Mellin's Food, when used at a 7% level in conjunction with milk resulted in hemoglobin values which were only slightly below the optimum. Orange juice and tomato juice, when fed at the level recommended for infants, had no effect on hemoglobin production, and the autolyzed yeast addition resulted in only slightly higher values.

The addition of iron and copper in the amounts shown to be effective in increasing the hemoglobin formation in rats was without significant influence in infants. Egg yolk in the amounts fed produced no beneficial effects on the hemoglobin in the infants studied; liver increased the hemoglobin values only slightly. These results would seem to be due to the fact that the values observed, 11 gm. to 13 gm. per 100 cc. of blood before the additions of egg yolk and liver are very nearly optimum for the age group under observation.

The highest hemoglobin values observed in the infants studied were those receiving wheat embryo extract. This was contrary to the findings with rats, for in these the wheat embryo extract appeared to have no effect in increasing the hemoglobin values. A possible explanation for the results with infants is the better absorption of certain hemapoietic stimulating substances. This however was not tested.

5351

Treatment of Experimental Trichinosis in Rabbits with Neutroflavine.

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In connection with tests of certain drugs in treatment of experimental trichinosis in rabbits, two series of experiments were carried

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† Junior Zoologist, Zoological Division, Bureau of Animal Industry.

out with neutroflavine. After determining that neutroflavine is well tolerated by normal rabbits which received 10 daily intravenous injections of 1.5 cc. of a 1% aqueous solution of this drug, the writers tested neutroflavine in cases of experimental trichinosis in rabbits as follows:

Series 1.—Each of 3 rabbits was fed about 8,000 decapsulated larvae of *Trichinella spiralis* obtained by digesting the muscles of a trichinous rabbit in an acidified solution of scale pepsin U. S. P. Two of the rabbits were given 12 daily injections of about 1.5 cc. of a 1% aqueous solution of neutroflavine, beginning on the seventh day after artificial infection. The third rabbit remained untreated. The first 7 treatments given to rabbit No. 1 were by the intravenous route, and the remaining 5 treatments were administered intramuscularly. Rabbit No. 2 received 12 consecutive injections intravenously.

The 3 rabbits were killed 25 days after experimental infection and examined for the presence of trichinae. Fourteen microscopic preparations were made from each of the following muscles: Tongue, masseter, diaphragm, intercostals, and thigh. The muscle preparations examined were fairly uniform as regards the bulk of muscle tissue in each preparation, and it is believed that the comparative larval counts, obtained in the various preparations examined, afford a good basis for judging the comparative degree of infection in the various rabbits involved in these experiments. Previous unpublished observations by the senior author, showed conclusively the reliability of this method for estimating the intensity of an infestation with *Trichinella spiralis* in rabbits. The total number of larvae counted in 14 preparations of each of the above mentioned muscles in the 2 treated and in the untreated control rabbit are given below:

NUMBER OF TRICHINAE FOUND IN SERIES 1.

	Tongue	Masseter	Diaphragm	Intercostals	Thigh	Total
Rabbit 1 (7 intravenous and 5 intramuscular injections)	5	9	5	0	1	20
Rabbit 2 (12 intravenous injections)	0	0	0	0	0	0
Rabbit 3 (Untreated)	69	51	34	5	5	164

Series 2.—Ten rabbits were used, of which 6 were treated with neutroflavine and 4 were kept as controls. Each rabbit was fed a quantity of trichinous rabbit meat containing approximately 2,400 encysted larvae. Treatments were begun 7 days after the meat was fed and continued for various periods. Each treatment consisted of an intravenous or an intramuscular injection of about 2 cc. of a 1% aqueous solution of neutroflavine. The results obtained were essentially similar to those of Series I.

Summary.—The data show that injections of neutroflavine into rabbits experimentally infected with *Trichinella spiralis* produced what appears to be a marked diminution in the number of larvae or the total destruction of the larvae. Presumably the larvae were destroyed in the circulation by the drug, since the best results were obtained with 10 to 12 consecutive daily intravenous injections of neutroflavine at a stage of the disease when the larvae are most abundant in the circulation.

5352

Influence of Morphine on Intestinal Activity in Experimental Obstruction.

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Two factors have previously been emphasized by this laboratory as contributing to late diagnosis in acute bowel obstruction, absence of local physical findings[1] in simple obstruction and mistrust in the value of enemas as a diagnostic criterion.[2] We here emphasize a third factor, namely, that in a large number of cases of intestinal obstruction at the University Hospital the administration of morphine has been responsible for delay in diagnosis.

This study is concerned with the effect of morphine in experimental intestinal obstruction, with particular reference to whether or not the sentinel warning of audible peristaltic rushes is ablated by the use of morphine.

* This work was supported by Grant 188 allowed by the Committee on Scientific Research of the American Medical Association.

[1] Wangensteen, O. H., and Lynch, F. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 674.

[2] Wangensteen, O. H., and Goehl, R. O., *Arch. Int. Med.*, 1930, **46**, 669.

It has been indicated that the response of the human intestine and that of experimental animals is not the same to injections of pituitary extract.[3] In this study preliminary tracings on 2 normal dogs and 8 hospital patients with ileostomy or colostomy, fistulous openings were obtained both before and after administration of morphine. This was done to make certain that comparable responses were obtained in the normal intestine of the dog and man after morphine administration.

Ten dogs were operated upon and intestinal obstruction of the simple variety was established in the lower ileum by severing the gut and inverting the ends. One to 6 days later tracings were made by inserting balloons mounted on rubber catheters into the obstructed ends of the bowel. The abdominal wall was then closed around the protruding catheter with clips. The catheters were attached to water manometers which recorded the findings on the revolving smoked drum. Morphine sulphate in 20 mg. doses was administered intravenously. In some cases the doses were repeated in larger amounts. In addition to the kymographic tracings auscultatory observations over the abdominal wall with a stethoscope were made both before and after administration of morphine. In a few instances the balloon on the catheter was placed in the end of the proximal bowel and an encircling ligature was placed around the bowel and catheter and the end of the bowel further invaginated by a pursestring and a few interrupted sutures. The distal bowel was treated similarly and both catheters were brought out through stab wounds. In 2 instances after the intestinal obstruction had been established by severing and inverting the ends of the bowel, auscultatory observations alone were made without opening the abdomen to make tracings.

In the case of the hospital patients the balloons were inserted directly through the ileostomy and colostomy openings into proximal and distal segments of the bowel, and 10 mg. of morphine sulphate were administered intravenously.

In the normal bowel of 8 human patients and 2 dogs the results following morphine injection uniformly indicated an almost immediate increase in intestinal tone followed by an increase in peristaltic activity. The response was more marked in the small bowel tracings than in the large bowel. These observations are in complete accord with the recently published work of Plant and Miller[4] and Gruber[5] and his associates.

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[3] Carlson, H. A., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 777.

[4] Plant and Miller, *J. Pharm. and Exp. Therap.*, 1926, **27**, 361.

[5] Gruber, Greene, Drayer and Crawford, *J. Pharm. and Exp. Therap.*, 1930, **38**, 389.

In the 10 dogs with simple obstruction of the bowel the response to morphine as shown in the tracings was essentially the same as in the normal bowel. Stethoscopic examination of the abdomen after administration of morphine revealed not only a persistence of the loud intestinal noises found previous to morphine administration, but there appeared to be an actual increase in loudness and frequency of peristaltic rushes. Subsequent doses of morphine resulted in similar but less forceful responses. In one instance the administration of 20 mg. morphine sulphate was followed by a prompt response within 2 seconds. In other instances the response came in 4 seconds and in one it was delayed 14 seconds. Within 3 minutes there were heard by stethoscope at intervals of 15-20 seconds powerful peristaltic rushes with a constant gurgling, churning, peristaltic activity between them. In one dog these sounds were so loud as to command one's immediate attention at a distance of 3 feet away with the unaided ear. Within 20 minutes these loud peristaltic rushes reached a frequency of about one every 5 seconds and by the end of one hour subsided to one every half to one minute apart. The less audible, more or less constant gurgling peristaltic activity persisted between the rushes. At the end of 2½ hours the gurgling peristaltic noises were still definitely heard. When a 50 mg. dose of morphine was repeated at this time, a similar response was evoked except that the rushes were not quite so loud nor did they persist as long. This was confirmed by kymographic tracings in which a second and third dose of morphine of 50 and 100 mgs. respectively provoked successively less marked responses.

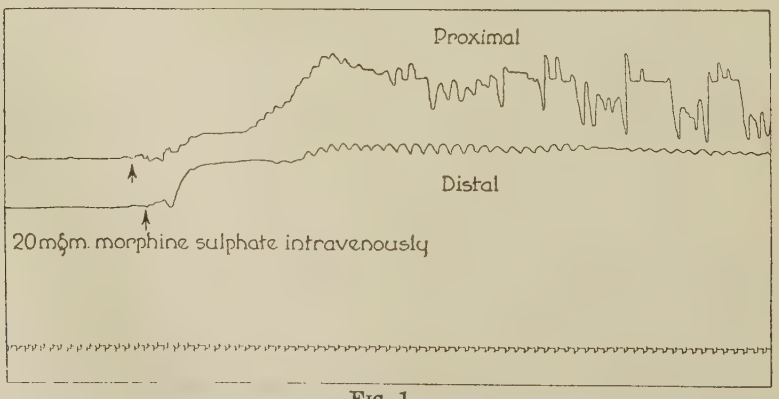

FIG. 1.

Ileum obstructed 5 days. Dog 32, 9-4-30.

The above figure shows a kymographic tracing of the lower ileum obstructed 5 days. The upper curve represents the proximal obstructed segment; the lower curve, the distal segment. The arrows indicate the time of injection of the morphine.

Since this study was started the abdomens of 5 patients with acute intestinal obstruction have been auscultated before and after the administration of morphine preliminary to operation. In all instances the loud intestinal gurgling noises have persisted after the morphine was given. In the diagnosis of obstruction the concomitant occurrence of the peristaltic rushes at the height of the pain is of great importance. When the pain is assuaged the interpretation of the significance of the persistent intestinal noises, which are not silenced by morphine, is more difficult to evaluate.

Conclusions.—Morphine increases the intestinal tone and peristaltic activity of the obstructed intestine of the dog. Loud intestinal borborygmi which may be heard with the stethoscope early in intestinal obstruction are not silenced by the administration of morphine.

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Influence of Hyperventilation on Experimentally Produced Gastric Secretion.

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During the course of an investigation by one of us[1] of the effect of vagal stimulation on the production of gastric secretion, it was observed that artificial ventilation considerably diminished the volume of the secretion. To ascertain the factors involved in this effect the following experiments were performed on dogs anesthetized with a chloralose and urethane mixture given intravenously.

1. Gastric secretion was obtained by vagal stimulation in the neck. The right and left nerves were stimulated alternately for 10 minutes each throughout the experiment. 2. Hyperventilation was applied. 3. Hyperventilation was continued at the same rate using an artificial air-carbon-dioxide mixture.

A study was made of the gastric juice, the collecting tubes being changed every 10 minutes. Total and free acid were determined by titration, and total chlorides by the method of Wilson and Ball.[2]

[1] Vineberg, A. M., *Am. J. Physiol.*, in press.

[2] Wilson, D. W., and Ball, E. G., *J. Biol. Chem.*, 1928, **79**, 221.

The arterial (carotid) plasma carbon dioxide content was determined by the volumetric method of Van Slyke, and the plasma pH by the colorimetric method of Cullen.[3]

While the vagi were stimulated the volume of gastric secretion rose gradually over a period of 2 hours to a value of from 8 to 10 cc. in 10 minutes. The free and total acid also rose, the former to a concentration of 0.24 mg. %. The initial arterial CO_2 content was 34.8 vols. %, and the pH 7.27; both somewhat low, due probably to primary ether anaesthesia. After this period the CO_2 content was 43.3 and the pH 7.33. Artificial hyperventilation was then applied at the rate of 64 per minute, and later at 84 per minute, the respiratory rate up to this point having been 12. The sample immediately following this showed a large increase in volume but no appreciable change in free or total acidity. This was probably due to a mechanical expression of previously-formed secretion. Subsequently over a period of 80 minutes the volume and the free and total acid were markedly diminished, the volume to an average value of 3.5 cc. in 10 minutes, the free acid to a concentration of 0.10 mg. %. The total acid ran parallel to the free acid and the total chlorides showed no appreciable change. Blood taken during this period showed a CO_2 content of 25.4 vols. % and a pH of 7.70.

After this sample was taken, air containing 8% carbon dioxide was given, the respiration being kept constant at a rate of 84 per minute. There was a latent period of 10 minutes, after which the volume showed a distinct rise to an average value of 5.5 cc. in 10 minutes. The free acid rose to an average value of 0.27 mg. %. The total acid rose in a parallel manner and the total chlorides showed a slight increase. The blood showed a CO_2 content of 41.4 volumes % and a pH of 7.17. The changes in the gastric secretion were maintained over a period of 35 minutes, at which point the experiment was discontinued.

Similar results have been observed in a number of experiments, but on 2 occasions the gastric secretion under hyperventilation showed an initial slight fall in volume and free and total acid, rising later to control values. The CO_2 content in both these experiments was at the extremely low value of 9 volumes %, whereas in the typical experiments it was about 25 volumes %. In this preliminary communication we hesitate to venture an interpretation of these findings, but it seems to us that the parallelism between the CO_2 content of the plasma and gastric secretion may be of significance in the light of Maly's old theory of gastric secretion.

[3] Cullen, G. E., *J. Biol. Chem.*, 1922, **52**, 501.

5354

III. Vitamin A Deficiency on Concentration of Sugar, Alkaline Reserve, and Glycogen Content of the Liver.*

BARNETT SURE AND MARGARET ELIZABETH SMITH.

From the Laboratories of Agricultural Chemistry and Home Economics, University of Arkansas, Fayetteville.

We have employed 24 animals, 29 to 55 days of age, that were transferred from our stock diet No. 2[1] to vitamin A deficient rations. The experimental basal ration used in this work had the following composition: Casein (hot-alcohol extracted) 20; Northwestern yeast 10; salts No. 185,[2] 4; lard 2; dextrin 64, irradiated for 30 minutes to insure an adequacy of vitamin D. In a number of experiments the lard in the ration was replaced by 1 to 2% of butter fat, in order to prolong the experimental period, so that the animals would be suffering from an insufficiency of vitamin A rather than from complete depletion of this dietary factor. This was done to prevent sudden death from pneumonia which occurs when all traces of vitamin A are removed from purified diets. The period of experimentation ranged from 80 to 150 days.

The methods used for the determination of blood constituents have been reported in previous publications.[3, 4] The same technique was employed in our studies of vitamins D and G deficiencies, the results of which follow in the subsequent articles. In this study, as well as in those that follow, daily records were kept of food and water intake in the case of all animals, and the blood sugars were determined twice weekly. In addition, determinations of specific gravity were made at each bleeding, in order to obtain information on blood concentration.

Our results show that in various stages of vitamin A deficiency characterized by the severity of eye lesions, there are no significant changes in the concentration of true blood sugar. The figures approximate those found in animals on satisfactory diets.[3] In a good many instances the concentration of apparent sugar is considerably higher in the pathological animals than in the controls. The results of work in progress will reveal whether such high values for the

* Research paper No. 194, Journal series, University of Arkansas.

1 Sure, B., *J. Biol. Chem.*, 1928, **76**, 728.

2 McCollum, E. V., and Simmonds, N., *J. Biol. Chem.*, 1918, **33**, 63.

3 Sure, B., and Smith, M. E., *J. Biol. Chem.*, 1929, **84**, 727.

4 Sure, B., and Smith, M. E., *J. Biol. Chem.*, 1929, **82**, 307.

latter constituent may be due to non-protein nitrogen contained in the non-reducing sugars.

We have not encountered the acidosis in vitamin A deficiency as we have in vitamin B deficiency.[3] Only 2 animals showed considerable reduction in the carbon dioxide volume capacity, one (28%) with mild ophthalmia, and the other (27.5%) with advanced eye lesions.

Expressed as milligrams of glucose per 100 gm. of liver the vitamin A deficient animals were found to contain 145.5 mg. glycogen which shows no noteworthy deviation from the figure of 138.3 mg. for our control adult animals.

Autopsy examinations revealed gross pathological changes in the respiratory tract of all the animals, either pus in the bronchi, hemorrhages in the lungs, or pneumonia, bronchial pneumonia being the most common. The following fact has, however, become clear on the careful study of the records of the 24 animals studied that, although there is a reduction in the food intake in vitamin A deficiency in most of the animals, it is not as pronounced as in vitamin B deficiency, complete anorexia being rather infrequent. It became also apparent that there was no specific relation between the water and food intake in advanced stages of vitamin A deficiency. It was observed, however, that in a good many instances excessive volumes of water, as much as 15 to 25 cc. daily, were consumed when the daily food intake was not more than 1 to 4 gm.

5355

IV. Vitamin D Deficiency on Concentration of Sugar, Alkaline Reserve, and Glycogen Content of the Liver.*

BARNETT SURE AND MARGARET ELIZABETH SMITH.

From the Laboratories of Agricultural Chemistry and Home Economics, University of Arkansas, Fayetteville.

In this study we have employed 25 animals transferred from our stock diet No. 2[1] to Steenbock and Black ricketic ration No. 2965.[2] Thirteen of these rats, which served as controls, received the same diets supplemented with vitamin D. The latter was supplied either

* Research paper No. 195, Journal series, University of Arkansas.

1 Sure, B., *J. Biol. Chem.*, 1928, **76**, 728.

2 Steenbock, H., and Black, A., *J. Biol. Chem.*, 1925, **64**, 263.

by irradiation of that ration or by the addition of 6 drops of cod liver oil daily per animal. At the end of the experiment a line test[3] was made on each animal.

Five out of the 13 animals showed absolutely normal calcification. The rest of the 8 controls showed a slight deviation from normal calcification, and yet could not be considered as cases of even mild rickets, judging from the line tests. Exposing some of these animals to light did not improve their calcification, because we encountered better calcification on some animals that received the irradiated ration and cod liver oil in the dark. We found only 2 cases out of 12 with severe rickets, the other 10 showing a narrow line of calcification which places them in a group of moderate rickets, as evidenced by the line tests.

An analysis of our data shows that there are no demonstrable changes in true blood sugar, alkaline reserve, or glycogen content of the liver in moderate or severe rickets, after a comparison is made between ricketic and control animals. The liver glycogen is higher than in our animals depleted of vitamin A, the average figure for the control being over 200 mg. expressed as glucose per 100 gm. of liver. This higher figure is undoubtedly due to the fact that the animals were younger, corresponding to our control weaned rats which show a figure of 164.8 mg.

We encountered high figures for apparent sugar both in control and pathological animals, the maximum average figure for non-sugar reducing substances in the control group being 48 mg. %, and 54 mg. % in the pathological group. When it is considered, however, that the Steenbock-Black ration No. 2965 is not an entirely satisfactory diet for optimum physiological function, it is not surprising to find high values for apparent sugar (166 to 178 mg. %) on that diet even if it is supplemented with vitamin D. The high figure of such blood constituent in pathological animals, therefore, cannot be attributed to a deficiency of vitamin D.

A detailed examination of the records of 25 animals leads us to conclude that vitamin D deficiency has no influence on food and water intake.

[3] Shipley, P. G., Park, E. A., McCollum, E. V., Simmonds, N., and Parsons, H. T., *J. Biol. Chem.*, 1921, **45**, 343.

5356

V. Vitamin G Deficiency on Concentration of Sugar, Alkaline Reserve, and Glycogen Content of the Liver.*

BARNETT SURE AND MARGARET ELIZABETH SMITH.

From the Laboratories of Agricultural Chemistry and Home Economics, University of Arkansas, Fayetteville.

In this investigation we have employed a total of 62 animals, 50 pathological and 12 control. The experimental period ranged from 60 to 220 days. The avitaminosis was produced on the dietary regime described by one of us (B. S.) elsewhere.[1, 2] Dermatitis was produced in 24 of these rats. To summarize our results neither arrest of growth nor loss of body weight nor accompanying dermatitis, associated with vitamin G deficiency, had any influence on apparent or true sugar or alkaline reserve. There was a reduction in the glycogen content of the liver in animals that had lost considerably in weight during periods of inanition.

In connection with our studies on the biochemistry and pathology of vitamin G deficiency we wish to point out at this time that from observations made on a total of 125 animals on various vitamin G deficient diets it became apparent that in the majority of animals that developed skin lesions dermatitis preceded the arrest of growth by 20 to 50 days, remarkable growth together with severe dermatitis being quite common. We have also encountered severe skin lesions in a number of positive controls (receiving 10 % autoclaved Northwestern yeast as a source of vitamin G) which showed a growth performance far superior to the Donaldson standard. On the other hand, in our experience, the dietary regime of Sherman and Sandels[3] produced a failure of growth, finally resulting in collapse, unassociated with dermatitis, only 2 animals out of 36 having developed skin lesions. We, therefore, conclude that the growth-promoting and anti-dermatitis factors associated with the "so-called anti-pellagric vitamin G" are not synonymous.

* Research paper No. 196, Journal series, University of Arkansas.

1 Thatcher, H. S., and Sure, B., *Archiv. Path.*, in press.

2 Thatcher, H. S., Sure, B., and Walker, D. J., *So. Med. J.*, 1930, **23**, 143.

3 Sherman, H. C., and Sandels, M. R., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 536.

5357

Increased Resistance of Rachitic Rats Exposed to Sunlight Through Vita Glass.*

JOHN R. ROSS AND ELIZABETH CHANT ROBERTSON.
(Introduced by F. F. Tisdall.)

From the Research Laboratories of the Sub-Department of Paediatrics, University of Toronto, and the Hospital for Sick Children, Toronto, under the direction of Alan Brown, M.B.

Each week albino rats, 4 weeks old, were separated as evenly as possible into 2 groups. The diet of the breeding rats had previously been regulated, so that it contained no excess of vitamin D. The young rats were put on Steenbock's rachitogenic diet 2965.[1] One group was exposed to the sun under plain or ordinary window glass, and the other under Vita glass of the same thickness, for 2 hours daily, except Sunday, from 11 A. M. to 1 P. M. for 4 weeks. Groups of rats were started once a week during May and again in the last 2 weeks of August and the first week in September. For their daily exposures, the rats were put out on the roof of a 5 story building in round wire cages covered by a square glass box, open at the 2 ends. An extra empty ordinary glass box was fitted closely at one end and a wooden cover over an electric fan at the other, so that the rats in the center cages were protected from any unrefracted sunlight, although when the fan was turned on, there was a good current of air over the animals. At first the rats were put out under a box covered on all sides except the bottom with glass, with no provision for the circulation of air, but on a warm day many of them died after 40 minutes' exposure to the sun. After the fans were installed this trouble was not encountered.

After 4 weeks, 4 rats were killed in each experimental lot (2 plain glass and 2 Vita glass) to determine the degree of rickets. The blood phosphorus was estimated by the method described by Tisdall,[2] and the bone ash percentage by that of Bethke, Steenbock and Nelson.[3] The results are shown in Table I. The Vita glass prevented the development of rickets.

The remaining rats were starved for about 20 hours, then weighed and put in individual cages kept on wide wooden shelves, so that

* For the details of the technique of these experiments see the earlier paper by the same authors, PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 999.

1 Steenbock, H., and Black, A., *J. Biol. Chem.*, 1925, **64**, 263.

2 Tisdall, F. F., *J. Biol. Chem.*, 1922, **50**, 329.

3 Bethke, R. M., Steenbock, H., and Nelson, M. T., *J. Biol. Chem.*, 1923, **58**, 71.

there was no possibility of any infected feces dropping into the cages below. The floors of the cages were sterilized 3 times a week. There was considerable variation in the weights of the rats in both groups, but those exposed under Vita glass weighed on the average slightly more (64 gm.) than those exposed under plain glass (59 gm.) by this time. Every rat was then given 1/20 of a cc. of an 18 hour culture of *Salmonella muriotitis* on a small piece of dried bread, except in the 2 experiments noted in Table II. After they were infected the exposures to sunshine were continued for another 4 weeks.

TABLE I.

Exp.	Plain Glass			Vita Glass		
	Blood P. mgm. per 100 cc.	Bone Ash %	X-ray Showing Rickets	Blood P. mgm. per 100 cc.	Bone Ash %	X-ray Showing
1	—	—	—	4.2	53.2	No rickets
2	1.6	24.3	Marked	3.0	40.1	Slight rickets
3	4.8	24.7	"	5.7	44.8	No rickets
4	3.2	31.4	Moderate	5.7	38.9	Slight rickets
5	1.7	30.9	Marked	—	44.3	No rickets
6	—	42.3	Moderate	4.0	50.6	" "
7	—	37.0	Slight	—	42.1	" "

TABLE II.

Exp.	Exposure to Sun Commenced	Plain Glass		Vita Glass	
		No. of Rats	No. of Survivors	No. of Rats	No. of Survivors
1*	May 5	2	1	5	3
2	May 12	6	2	4	3
3	May 19	7	4	8	7
4†	May 27	6	0	7	2
5	Aug. 13	4	0	6	3
6	Aug. 29	5	2	8	4
7	Sept. 4	2	0	3	3
Total		32	9	41	25

* Dose—0.01 cc. † Dose—0.1 cc.

As shown in Table II, considerably more of the rats exposed under Vita glass survived the infection; 25 of 41 such rats or 61% survived. Of those exposed under plain glass, only 9 of 32 or 28% survived. The survivors all lived at least 28 days after eating the infected bread. All of the rats which died yielded *S. muriotitis* from the heart's blood at post mortem, and showed characteristic gross pathological lesions. Only 3 of the rats which died lived more than 16 days after the infection.

Blood cultures were made on 50 rachitic rats which had not been infected, and only 2 of these showed *S. muriotitis*. Of 84 fecal cultures on similar rats 3 yielded *S. muriotitis*. In 9 rats the fecal cultures were repeated 3 times at intervals of a few days and were uniformly negative. A series of rachitic rats (McCollum's diet 3143[4] for 4 weeks) were fed ½ cc. of the *S. muriotitis* culture and killed at daily intervals. The organism was recovered from the mesenteric glands, liver and spleen in 48 hours and from the blood stream in 5 days.

Using the results in Tables I and II, it appears that the difference in the survival rate between the Vita and the plain glass rats in each experiment corresponds roughly with the difference in the bone ash between the corresponding rats. The difference in the bone ash indicates the difference in the degree of rickets. In other words, it seems probable that the degree of resistance varies approximately with the degree of rickets present.

Summary.—Of 41 rats fed a rachitic diet and exposed to sunshine through Vita glass, 61% survived a *per os* enteriditis infection, as compared with 28% of 32 similar rats exposed to sunshine through plain or ordinary glass.

5358

Effects of Ethyl Alcohol on Red Blood Cells.

JOHN W. WILLIAMS. (Introduced by C. W. Duval.)

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The observations present the microscopic changes occurring in human red blood cells when subjected to different dilutions of alcohol in saline. Approximately 50 specimens of blood from various patients were studied and the results obtained conform as a whole. Studies were made by the hanging drop method. Cells deposited in the serum of clotted blood were examined within 4 hours after withdrawal and whole blood was examined immediately following withdrawal. The red cells were diluted so that the suspension contained approximately 4000 per cu. mm.

When the red blood cells were found to be in rouleux formation the alcohol dilutions overcame this formation more quickly than

[4] McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A., *J. Biol. Chem.*, 1921, **47**, 507.

when normal saline was added, and the rapidity of this change in all cases depended upon the concentration of the alcohol.

In one series, various iso-osmotic solutions of alcohol and sodium chloride were employed. In 0.1% alcohol the cells were more rounded and regular and this was more apparent in 0.5% alcohol. In the 1% alcohol, granules were extruded from the cells, coincidently they lost their pigment, became swollen and faded away as shadows.

In the other series, alcohol was diluted with normal saline. In 0.2% (approximately 1.0016 × iso-osmotic) alcohol a cupped appearance was noted and a few of the cells buckled in and bent upon themselves; the inner edges of the cups showed in a few instances serrations and the margins of the cups were more refractile and apparent and the centers clearer. In 0.5% (approximately 1.003 × iso-osmotic) alcohol the irregularity of the cells was more marked. In this concentration numerous cells possessed very clear centers shaped like triangles and slits, while a few appeared small and round and no longer presented the cup effect. In 1% concentration (approximately 1.008 × iso-osmotic), many cells gave the impression of rubber tires with a clear refractile rim, and many appeared larger than the circular cells in normal saline. In 5% alcohol (approximately 4 × iso-osmotic) the cells were largely circular while in 10% concentration (approximately 7.3 × iso-osmotic) they became smaller, crenated, the cup effect disappeared and the cells appeared irregular and shrunken. In the 15% concentration (approximately 19.54 × iso-osmotic) most of the cells were irregular and crenated, but a few were small, uniform in consistency and clear. In 20% alcohol (approximately 13.8 × iso-osmotic) they were finely granular with a fuzzy periphery but uniform in size, some extruded their granules and became clear and pale and in some cases larger. In the 25 to 30% concentrations (approximately 17 and 20 × iso-osmotic) the cells lost their granules, swelling in most cases, and faded away as shadows. When the majority of the cells examined were already crenated, there was a tendency for their granules to become smaller and for the cells to become more regular in the 0.2%, 0.5% and 1% alcohol concentrations. In 5 and 10% alcohol they were irregular and crenated but apparently clearer than those in saline. In the 20% concentration the cells began to clear and become round, and in the 30% they faded away as shadows.

When 95% alcohol was added directly to the red cells, many, maintaining their cup effect, faded away as shadows, while others maintained their original size or became even larger, faded away,

resulting in clumps of granules. This granular residue collected in masses which in many cases seemed located in the framework of the original cells.

In the test tube, complete hemolysis was effected in each case in a 30% concentration of alcohol in normal saline. The red cells showed no greater fragility to 20% alcohol in hypotonic solutions of sodium chlorides of varying concentrations than to hypotonic solutions of sodium chloride in varying concentrations.

This work was originally intended to correlate the microscopic changes in red blood cells with the percentages of alcohol present in blood of man coincident with the feeling of well being and the condition of stupor. However, the microscopic changes which take place in these percentages are not sufficiently marked to assume that they might not take place physiologically as a result of physico-chemical changes occurring normally in circulating blood. The results noted in iso-osmotic solutions of alcohol and sodium chloride indicate that tonicity of solution is not the only factor which plays a part in the case of alcohol. In the solutions studied at least 2 forms of crenation were noted, the one in the solution of iso-osmotic normal saline, and the other in the dilutions of higher tonicity as, for example, the 10% alcoholic solution. In the first type the cells were irregular in outline, coarsely granular and often larger than the unaffected cells and their periphery was studded with projecting knobs. In the second type the cells were shrunken, finely granular, uniform in size and shape and their periphery was studded with fine spicules. Whether this later type of crenated cell is characteristic of hypertonic solutions cannot as yet be definitely stated. The disappearance of the cup effect in the red cell is followed in the hypertonic solutions by this crenated form, and it might be assumed that the shape of the crenated cell is the result of the filling of the cup with the cellular contents normally present at the rim. It is hoped that further work will clear up these details.*

* I wish to thank the Touro Infirmary and students of the sophomore class for the blood specimens which made this work possible.

5359

Glycogen Formation in the White Rat After Oral Administration of Xylose.

MABEL M. MILLER AND HOWARD B. LEWIS.

From the Laboratory of Physiological Chemistry, Medical School, University of Michigan, Ann Arbor.

Recent investigations on the preparation of the pentose, xylose, have made this sugar, formerly one of the rare carbohydrates, available at a very moderate price. It has been suggested that xylose may be utilized in human nutrition as a "non-fattening sugar." The renewed interest in xylose occasioned by its ready availability in pure form again raises the question of its rôle in nutrition. Although most of the literature would indicate that xylose ingestion does not lead to increased formation of liver glycogen, the question of its behavior in this respect is still an open one. We have accordingly studied the rate of absorption of xylose and the formation of glycogen from it in the young white rat.

The xylose was prepared from cottonseed hulls and was given to us by the Bureau of Standards and by Dr. J. L. Kassner of the University of Alabama, to whom we take this opportunity to express our indebtedness. The material as received was recrystallized from alcohol, dried at 40° in the oven and then in a desiccator for several weeks. The method of study was that of Cori as previously used in this laboratory for the study of the absorption of and glycogen formation from amino acids.[1]

The results are presented in tabular form and require little comment. After the absorption of xylose over periods of 1, 2 and 3 hours, there was no significant change in glycogen content of the liver or the entire body (Groups 2, 3, 4) as compared with the control fasted animals (Group 1). Glucose administered in amounts comparable to xylose resulted in a marked deposition of glycogen after an absorption period of 3 hours (Group 5). Since, however, glucose was absorbed much more rapidly than was xylose, it seemed possible that glycogen formation might not result from *the absorption of glucose in amounts comparable to the amount of xylose actually absorbed* as shown by the experimental data. Accordingly a second group of glucose controls (Group 6) received an amount of glucose such that the amount absorbed (47.4 mg. per

[1] Wilson, R. H., and Lewis, H. B., *J. Biol. Chem.*, 1929, **84**, 511; 1929-30, **85**, 559.

TABLE I.

	No. of Animals	Absorption per hr. per 100 gm. rat Average mg.	Glycogen	
			Liver Average %	Entire Body Except Liver Average %
1. Fasting Controls	11		0.105 (0.074-0.170)	0.051 (0.042-0.075)
2. Xylose, 1 hr.	6	29.3 (23-34.1)*	0.157 (0.092-0.262)	0.053 (0.045-0.061)
3. Xylose, 2 hrs.	6	39.9 (24-54)	0.129 (0.094-0.186)	0.061 (0.042-0.080)
4. Xylose, 3 "	12	46.7 (32-74)	0.134 (0.060-0.185)	0.059 (0.039-0.073)
5. Glucose, 3 hrs.	8	163.9 (144.4-205.4)	1.916 (1.149-2.997)	0.153 (0.095-0.251)
6. Glucose, 3 " fed at level of xylose absorption	11	47.4 (41.8-54.1)	0.604 (0.459-0.725)	0.088 (0.075-0.101)

* The figures in parenthesis indicate the ranges observed in the individual experiments.

hour per 100 gm. of rat) over a period of 3 hours was similar to the amount of xylose absorbed in a like period (46.7 mg. per hour per 100 gm. of rat). A significant glycogen formation in the livers of this group was also observed, the average figure being 4 to 5 times greater than that of the control fasting rats or the rats receiving xylose. This would seem to indicate that xylose *in the amounts actually absorbed* should cause a significant deposition of glycogen if glycogen formation from it occurred readily. We must therefore conclude that xylose, under our experimental conditions, is not readily available for the formation of glycogen.

5360

Nature of the Agent Transmitting Leucosis of the Fowl.*

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From the Henry Phipps Institute, University of Pennsylvania, Philadelphia.

The studies of Ellerman and Bang,[1] Furth[2] have shown that the agent transmitting leucosis of fowls passes bacteria-tight Berkefeld

* This investigation has been supported by a Fund for the Study of Leucemia and Related Diseases.

† With the assistance of Charles Breedis.

1 Ellermann, V., The Leucosis of Fowls and Leucemia Problems, London, 1921.

2 Furth, J., *J. Exp. Med.*, 1931, **53**.

filters. In most instances the bulk of the transmissible agent was easily removed from the blood by a brief centrifugalization, suggesting a relation of this agent to cells such as has been observed with some viruses. Moreover the opinion of some that the transmission of Rous sarcoma is bound to cells[3] or cell-fragments that pass silicous filters[4] has induced me to investigate the relation of the principle transmitting leucosis to cells.

When diminishing amounts of whole blood or blood cells of leucemic fowls were injected into healthy fowls leucosis was usually not produced with amounts less than about 0.0002 cc. (in terms of the original whole blood) although this quantity of blood contained from 5,000 to 50,000 leucocytes. Therefore, should transmission depend on the presence of white cells a very large number of them would be required to produce leucosis.

Undiluted plasma, after recentrifugalization for about 8 minutes at about 2000 revolutions per minute, when placed in the counting chamber appeared cell-free. Though the plasma was in all but one experiment less active than the cell suspension in producing leucosis, amounts as small as those actually contained in the counting chambers (0.002 to 0.01 cc.) were in several experiments sufficient to transmit leucosis.

No cells could be shown in Berkefeld V and N filtrates of plasma. Even a very coarse non-bacteria tight filter resisting only an air pressure of about 190 mm. appeared to retain leucocytes completely. Since most of the leucocytes can be rapidly thrown to the bottom of a tube by spinning, absence of cells in the bottom layer of such material is sufficient evidence that the entire filtrate is either cell-free or that it contains cells in small number, insufficient to transmit lesions.

Very recently Jármai[5] observed that Zsigmondy-Bachman membranes with an estimated pore-size of about 20 to 100 millimicrons did not retain the transmissible agent of leucosis of fowls but that finer membranes retained this agent and proteins as well. The observations of Jármai as well as our own permit the conclusion that leucosis of the fowl may be transmitted by cell-free material and that its causative agent is filterable.

Filtrable agents of tumors are apparently distinguishable from those causing infectious diseases. The behavior of the filtrable agent of the leucosis of fowls seems similar to that of filtrable tumors. This separation is suggested by certain features that are

[3] Nakahara, W., *Jap. J. Exp. Med.*, 1928, **7**, 101.

[4] *Cf.* Teutschlaender, O., *Z. f. Krebsf.*, 1923, **20**, 43.

[5] Jármai, K., *Arch. f. dissensch. und prakt. Tierheilkunde*, 1930, **62**, 113.

probably common to the filtrable agents of tumors and of leucosis although they have not been satisfactorily investigated in this connection. These are as follows:

Resistance of certain individual animals to these transmissible agents is not based on immunological principles but appears to be governed chiefly by hereditary factors. Ellermann observed that fowls that resisted one inoculation might occasionally succumb to reinoculation. We have found that the blood of a fowl that had recovered from leucosis did not exert any protective action when inoculated simultaneously with leucemic blood into 5 fowls, for all of them developed leucosis about the same time as the corresponding controls.

Some fowls resist inoculation although they are given several hundred times the amount necessary to cause leucosis in susceptible individuals. In one passage, for example, 4 fowls were inoculated with 0.2 cc., 4 with 0.001 cc. and 4 with 0.00005 cc. of plasma (not cell-free). One of the fowls inoculated with 0.2 cc. resisted the disease although 2 of those receiving 0.001 cc. and one of those receiving 0.00005 cc. developed leucosis.

Filtrable tumors of the fowl unlike other filtrable virus diseases of the fowl could not be transmitted to other species of birds. The transmissible agent of filtrable tumors appears to be in large part attached to cells or to cell fragments.

These properties as well as the character of the disease produced (neoplastic growth) seem to justify a separation of the filtrable agents of tumors within the apparently heterogenous group of filtrable viruses and suggest the possibility that the agent of filtrable tumors is not a virus in the ordinary sense.

5361

Blood Lactic Acid and the Coronary Circulation.*

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Increasing information on the distribution of lactic acid in the body tissues has suggested that it plays a more complex rôle than has generally been suspected. Evidence was produced[1] to show

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[1] McGinty, D. A., *Am. J. Physiol.*, 1929, **88**, 312.

that brain tissue removes lactic acid from the blood under normal conditions and that lactic acid is added to the blood circulating through the brain during impaired oxidations induced experimentally. It is evident that this equilibrium between lactic acid absorption and outward diffusion must play a part in the acid-base balance of the blood as well as in the brain. Under conditions in which lactic acid is produced in excessive amounts, its removal from the blood is a matter of importance. Himwich and his collaborators[2] showed that excess blood lactic acid produced chiefly in muscles is carried to the liver and stored as glycogen to be converted subsequently into glucose. These findings have been confirmed and extended by Cori and Cori.[3] In an effort to find further sources of blood lactic acid or further means of its disposal, a study was made of the lactic acid content of arterial and coronary venous blood of the heart *in situ*.

Dogs were anesthetized either with morphine and urethane or with Gréhant's mixture of chloroform and alcohol. The chest was opened during constant artificial respiration and a cannula placed in the coronary sinus. Volume flow of coronary blood was recorded in most of the experiments. Simultaneous samples of arterial and coronary venous blood were analyzed for lactic acid, a difference of 3 mg. % or more being considered as of definite physiological significance.

In 118 out of 120 pairs of samples in 11 dogs under a variety of experimental conditions, coronary venous blood contained less lactic acid than arterial blood by amounts ranging from within the analytical error to 23 mg. %. No parallelism could be observed between the extent of absorption as shown by the arterial-venous differences in the coronary system and the general arterial lactic acid levels which ranged from well within normal limits to values exceeding 100 mg. %.

In 5 out of 7 observations with stimulation of the stellate ganglion followed by increased heart rate, blood pressure and coronary volume flow, arterial-venous differences fell from an average of 8.0 mg. % to 3.8 mg. %. Usually this was followed by recovery. In 2 experiments in which only a slight change in volume flow took place, no significant change in lactic acid absorption was observed.

Stimulation of the vagus, in 9 of 13 observations caused an increase in the arterial-venous difference from an average of 5.4 to

[2] Himwich, H. E., Koskoff, Y. D., and Nahum, L. H., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 347.

[3] Cori, C. F., and Cori, G. T., The Harvey Lectures, 1927-28.

8.6 mg. % during the fall in volume flow of blood. In 2 experiments no change occurred. A decrease in arterial-venous difference appeared in 2 observations in which blood samples were taken at least 2 minutes after the end of stimulation and much later than ordinarily.

In 2 experiments with rapid intravenous injection of large doses of pitressin, following which a considerable reduction in coronary volume flow occurred, an initial pre-injection absorption of 7.5 and 10.1 mg. % lactic acid was replaced by an outward diffusion of 4.1 and 4.2 mg. % respectively. On recovery absorption again took place.

In 4 experiments a reduction of arterial-venous difference occurred during the height of the pitressin effect on the heart, and in 3 observations with considerably smaller amounts of pitressin an increased lactic acid absorption was observed during the moderate fall in volume flow of blood.

The gradient of inward or outward movement of lactic acid between blood and heart muscle is dependent on the relative concentrations in the blood and muscle. The concentration in muscle may be regarded as being dependent on the state of equilibrium between disposal and production of lactic acid. With moderate changes in coronary volume flow during vagal and stellate stimulation and with small doses of pitressin, the rate of absorption of lactic acid is believed to be primarily dependent on the velocity of the blood through the heart capillaries. With more pronounced diminution in flow of blood with a subsequent reduction in oxidations in heart muscle, increased lactic acid production may reduce the gradient between blood and muscle or with larger injections of pitressin establish a reverse gradient from muscle to blood.

These experiments demonstrate that the heart under normal conditions must be regarded as one of the organs concerned with the removal of lactic acid from the blood. The fate of the lactic acid thus absorbed and its relation to oxidations in heart muscle is under investigation.

5362

Protein and Amino Acid Feeding upon Creatine Formation in Muscle, and Creatinine Elimination in Urine.

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It is generally believed that creatine is a tissue constituent with a special function and that it arises in the body as a result of a specific cellular demand for it. There is also much evidence to show that it may also be derived from certain precursors, *e. g.*, arginine,[1] glycine,[2] cystine,[3] and histidine,[4] in the diet.

About 3 years ago, using young rats and mice, it was observed in this laboratory that the feeding of d-arginine monohydrochloride gave slightly larger increases in muscle creatine than creatine itself, when each of these substances formed 5% of the standard casein diet. The possibility that this amino acid was one of the precursors of creatine in the animal body was suggested. With positive evidence for the other amino acids mentioned above, a systematic study was begun to determine the influence of feeding proteins, amino acids, and related substances upon creatine formation in the muscles, and creatinine elimination in the urine, the results of which are presented below.

Young rats were placed on Sherman's Diet B for a period of 10 days after weaning. Amounts of purified amino acids up to 1.5 gm. were fed either as such or mixed with a small amount of the stock diet. In other experiments casein or edestin were fed. At the end of 17 to 48 hours the animals were killed and the muscle creatine determined by the method of Rose, Helmer and Chanutin.[5] The litter mate control animals received no amino acid or protein supplement. The average creatine content of the muscles of 118 control rats was 0.40%. The average results obtained are given in Table I.

The effect of amino acid feeding upon creatinine elimination in the urine was next studied with the hope that further light might be thrown upon the origin and metabolism of creatine. Rats weighing between 200 and 300 gm. were used. They were fed on Sher-

[1] Knoop, F., *Z. physiol. Chem.*, 1910, **67**, 489.

[2] Brand, E., Harris, M. M., Sandberg, M., and Ringer, A. I., Abs. 13th International Physiol. Congress, Boston, August, 1929.

[3] Harding, V. J., and Young, E. G., *J. Biol. Chem.*, 1920, **41**, xxxvi.

[4] Abderhalden, E., and Baudze, S., *Z. physiol. Chem.*, 1930, **189**, 65.

[5] Rose, W. C., Helmer, O. M., and Chanutin, A., *J. Biol. Chem.*, 1927, **75**, 543.

TABLE I.

Influence of Feeding Proteins, Amino Acids or Related Substances upon Creatine Formation in Rat Muscle.

Substance Fed	No. of Rats	Creatine, %	Increase Over Controls, %
Glycine	9	0.46	15.0
dl-Alanine	17	0.45	12.5
dl-Valine	6	0.54	35.0
d-Glutamic acid	8	0.48	20.0
l-Aspartic acid	10	0.47	17.5
l-Cystine	10	0.55	37.5
Histidine	4	0.49	22.5
dl-Phenylanine	5	0.49	22.5
l-Tyrosine	10	0.49	22.5
d-Arginine-HCl	11	0.51	27.5
l-Leucine	4	0.49	22.5
Choline-HCl	6	0.49	22.5
Glycocyamine	3	0.59	47.5
Casein	6	0.52	30.0
Edestin	6	0.52	30.0
Creatine	8	0.49	22.5

man's diet B and a 2-day control output of creatinine determined. On the morning of the third day, 1 or 1.5 gm. of a purified amino acid was fed as above, the stock diet removed, and replaced again when practically complete consumption of the acid was observed, and a second 2-day experimental creatinine output determined. All conditions were uniform throughout and the results obtained are due to the amino acid feeding. See Table II.

Further evidence confirming these results was obtained with several students and members of the laboratory staff, including the writer. The subjects were on a meat-free diet. The following supplements were taken in milk, 100 gm. casein; 100 gm. edestin; 5 gm. arginine-HCl; 8 gm. glycine; 10 gm. alanine. A rest period of at least 4 or 5 days always preceded the 24-hour experimental period. With one or 2 exceptions a definite and large increase in the daily elimination of creatinine was observed.

TABLE II.

Influence of Amino Acid Feeding upon Creatinine Elimination.

Amino Acid Fed	No. of Experiments	Average Increase in the Excretion of Creatinine %
Glycine	21	35.9
l-Aspartic Acid	11	14.2
d-Arginine-HCl	7	26.8
dl-Alanine	8	28.0
l-Cystine	8	28.1
dl-Valine	5	14.8
l-Tyrosine	8	30.7
d-Glutamic Acid	9	25.1
Histidine	6	19.5

Evidence was obtained which shows that the results of these studies were not due to a stimulation of the endogenous metabolism nor to the specific dynamic action of the proteins or amino acids.

The following conclusions were drawn: Both creatine and creatinine were formed, under the conditions of these experiments, as a result of an increased exogenous protein or amino acid metabolism per unit of time. Muscle creatine is an intermediate product and urinary creatinine a waste product of this metabolism. Evidence was obtained which showed that creatine may also have an endogenous origin from amino acids.

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Cure of Rickets by Water Soluble Extract of Yeast and Sodium Phosphate.

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Five albino rats, 28 days old, were removed from the mother to a dark room and placed in individual cages. The rats were of a known inbred stock of laboratory animals which had been under observation for 2 years. The animals were placed on Steenbock's Rachitogenic Diet No. 2965* and given distilled water only. This diet was continued for 30 days, when the animals were X-rayed, and all were found to have advanced rickets.

They were then returned to the dark room and fed the following diet: 960 gm. of Steenbock's Rachitogenic Diet No. 2965, thoroughly mixed with 40 gm. of $Na_2HPO_4 \cdot 12H_2O$. In addition one gram of a water soluble extract of yeast was added to each day's feeding. The water soluble extract of yeast was made by filtering cold double distilled water through brewers' yeast and evaporating the filtrate to a gum. This material was then dried by repeated trituration with absolute alcohol. The residue left after evaporation of the alcohol was combined with the original material and the whole powdered. This water soluble extract gave a negative test for sterols after it had been shaken out with chloroform and treated

* Steenbock's Rachitogenic Diet consists of: 76% whole yellow corn; 20% gluten; 3% CaCl; 1% NaCl.

with acetic anhydride and sulphuric acid (Liebermann-Burchard test.) But a sample of the whole yeast gave a decidedly positive reaction, and the water soluble extract of the yeast, to which one drop of Viosterol was added, also gave a strongly positive reaction.

The feeding of this latter diet was continued for 30 days, when the animals were again X-rayed, showing that the rickets had been cured.

This improvement was not due to time, since rats that continue to eat the rachitogenic diet for 60 days show skeletal changes that are at least as abnormal as those of the rats on the diet for 30 days, when examined by means of the X-ray.

In addition to this group of rats, other groups have been fed varying amounts of phosphate and the water soluble extract of yeast, with results that are analogous to those described.

Conclusion. It is clear that the addition of sterol free, water soluble, extract of yeast plus secondary sodium phosphate to a rachitogenic diet caused the disappearance of the rachitic skeletal changes in rats.

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Rôle of Certain Anaerobic Toxins in Pneumococcus Infection.

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The search for the factors responsible for the toxemia of pneumococcus infections is long and unsuccessful. The approach to this problem has been primarily by studies of the toxic substances which may be obtained from the pneumococcus *in vitro*. Following the intravenous injection of pneumococcus autolysates into guinea pigs, Rosenow[1] and Cole[2] early observed anaphylactic-like reactions which could not be correlated with the signs of toxemia in pneumococcus infections. Recently, Parker[3] described certain toxic substances obtained by the anaerobic autolysis of concentrated pneumococcus

* This study was aided by a grant from the Littauer Fund for Pneumonia Research at New York University.

1 Rosenow, E. C., *J. Infect. Dis.*, 1911, **9**, 190; 1912, **11**, 94, 235.

2 Cole, R., *J. Exp. Med.*, 1912, **16**, 644.

3 Parker, J. T., *J. Exp. Med.*, 1928, **47**, 531; 1929, **49**, 695; 1929, **50**, 161.

suspensions. The toxic principles in these anaerobic autolysates are capable of producing necrosis when injected intradermally, and death associated with marked pulmonary lesions when injected intratracheally into guinea pigs. These toxins are thermolabile and very sensitive to oxidation; they are species-specific and can be neutralized by heterologous anti-autolysate serum. Parker and McCoy[4] reported the production of potent antitoxic serum in horses.

With the hope of learning something concerning the nature of the factors causing the death of pneumococcus-infected animals as well as the possible therapeutic application of Parker's antipneumotoxic serum, an attempt was made to determine whether these toxins obtained *in vitro* played any part in the course of natural infection. It seemed that a specific rôle for the anaerobically produced toxins could be established only if the "antipneumotoxic" serum added to the ordinary antibacterial serum could save those animals in which antibacterial serum alone failed to avert death.

It is well known that when mice are infected with certain large doses of pneumococci, no amount of antibacterial serum can save them from death. Since it is suggested by some that death, here, might be due to the large amounts of liberated endotoxins which are not neutralized by the antibacterial serum, the following experiment was planned to test the effect of antipneumotoxin. Fifty mice were injected with large doses of Type II Pneumococcus. One series of mice was treated with therapeutic antibacterial serum only; another series with antipneumotoxin only, and a third series with both antibacterial and antipneumotoxic serum. The results shown in Table I indicate that the antipneumotoxin had no effect. The only conclusion that may be drawn, however, is that the anaerobically produced toxins probably do not play any part in the causation of death of mice infected with very large doses of virulent pneumococci.

Goodner[5] described the production of so-called "intradermal pneumonia", which consists of a local lesion associated with bacteremia and toxemia, following the intracutaneous injection of virulent pneumococci in rabbits; most of the rabbits, when untreated, die in 3 to 4 days. Since this infection in rabbits is more nearly related to the course of lobar pneumonia in man than is any other pneumococcus infection in animals, the effect of antipneumotoxin on its course was expected to yield valuable information. Rabbits were given an intradermal injection of 0.1 cc. of a 1-100 dilution of

[4] Parker, J. T., and McCoy, M. Van S., *J. Exp. Med.*, 1929, **50**, 103.
[5] Goodner, K., *J. Exp. Med.*, 1928, **68**, 2.

TABLE I.

Effect of Various Modes of Therapy on Massive Pneumococcus Infection in Mice.

Pneumococcus Type II Culture	Therapy Simultaneous with Culture	No. of Mice	No. of Survivals
cc. M.L.D.	*Antibacterial Serum*		
0.2 20 million	400 units	3	1
0.2 20 "	1000 "	3	0
0.4 40 "	400 "	3	0
0.4 40 "	1000 "	3	0
	"Antipneumotoxin"		
0.2 20 million	0.5 cc. (10,000 units)*	3	0
0.2 20 "	2.0 cc. (40,000 ")	3	0
0.4 40 "	0.5 cc. (10,000 ")	3	0
0.4 40 "	2.0 cc. (40,000 ")	3	0
	Antibacter.+Antipneumo.		
0.2 20 million	400 units 0.5 cc.	3	1
0.2 20 "	400 " 2.0 "	3	1
0.2 20 "	1000 " 0.5 "	3	0
0.2 20 "	1000 " 2.0 "	3	0
0.4 40 "	1000 " 0.5 "	2	0
0.4 40 "	1000 " 2.0 "	3	0

* One unit of antipneumotoxin is the smallest amount of serum required to protect a guinea pig (200-210 gm.) against one unit of toxin when the mixture is injected intratracheally. One unit of toxin is the amount which when injected intratracheally kills a guinea pig (200-210 gm.) in from 4 to 24 hrs., with typical symptoms and autopsy findings.

an 18-hour broth culture of fully virulent Type I Pneumococcus. One series of rabbits was treated with Type I antibacterial serum only, another with antipneumotoxin only, and a third series with both the antibacterial and antipneumotoxic serums. Some of the rabbits received the serums at 6 to 7 hours after infection, and others 24 hours after. The results are shown in Table II. The untreated rabbits and those receiving antipneumotoxin only, all died and within the same time. Of 4 rabbits treated with 300 units anti-

TABLE II.

Effect of Various Modes of Therapy on the Survival of Rabbits with "Intradermal Pneumonia".

Therapy	Hrs. After Intradermal Infection	No. of Rabbits	No. of Survivals
None	—	4	0
300 units Type I	7	2	0
	24	2	1
Antipneumotoxin 5 cc. (100,000 units)	7	2	0
	24	2	0
300 units Type I and Antipneumotoxin 5 cc. (100,000 units)	7	2	1
	24	2	1
	7	4	1
	24	4	0

bacterial serum only, one survived. Among the rabbits treated with 5 cc. (100,000 units) antipneumotoxin in addition to the 300 units of Type I antibacterial serum, 2 out of 4 survived in one experiment and only one out of 8 in another.

It is interesting to observe that at 6 hours after the intradermal infection, although the blood culture is strongly positive, the local lesion is either absent or characterized by very slight erythema only; the antipneumotoxic serum administered at this stage did not prevent its further development, nor did it have any apparent effect on the fully developed lesion when administered 24 hours after infection. In most of the rabbits treated at 6 to 7 hours and in some of those treated at 24 hours, the blood was sterilized and maintained sterile after the administration of the serum; in spite of the sterile blood culture, however, the local lesions grew progressively more marked and the temperature remained elevated. (Table III.) The rabbits died with an absent bacteremia and negative post-mortem culture of the hearts' blood. Death apparently was produced by the absorption of toxins from the local lesion, and these toxins were not neutralized by the presence of large amounts of antipneumotoxin, theoretically sufficient to protect 100,000 guinea pigs from death by one lethal dose of toxic autolysate.

TABLE III.

Protocols of Two Rabbits Treated with Antibacterial Serum and Antipneumotoxin.

Injections: Culture—intradermally—0.1 cc. of a 1-100, 18 hr. broth culture of fully virulent Type I Pneumococcus. Therapy—intravenously—300 units Type I serum and 5 cc. (100,000 units) antipneumotoxin, 6½ hours after culture.

Rabbit	Date	Hrs. after culture	Lesion-extent of oedema and erythema	Blood Culture		Temperature
				Plate organisms per cc. blood	Broth	
	11-7	6½	None	1200	Pos.	103.4°
	11-8	24	5×4 cm.	Neg.	Neg.	104.5°
21	11-9	52	11×6 "	"	"	105.0°
Temp. before culture	11-10	75	11×9 "	"	"	105.9°
11-6 4:00 P. M. 103.3°	11-11	96	11×11 "	"	"	104.8°
11-7 9:30 A. M. 102.5°	11-12	120	11×11 "			106.3°
	11-12	124	Dead	Culture of Heart's blood sterile		
	11-7	6½	None	1600	Pos.	103.4°
	11-8	24	6×5 cm.	8	Neg.	104.6°
22	11-9	52	8×5 "	Neg.	"	103.7°
Temp. before culture	11-10	75	10×7 "	"	"	105.6°
11-6 4:00 P. M. 102.8°	11-11	96	10×7 "			100°
11-7 9:30 A. M. 102.5°	11-12	110	Dead	Culture of Heart's blood sterile		(In shock)

Conclusions: Since the "antipneumotoxic" serum fails to modify the course of pneumococcus infection, as shown in the mice and rabbit experiments, it seems fair to assume that the anaerobically produced toxins are probably products primarily of the enzymatic changes occurring in *in vitro* autolysis, and play no part in the course of natural infection.

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Decremental Conduction in the Human Heart.

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Decremental conduction appears to have been demonstrated in the compressed or otherwise depressed mammalian auricular muscle (Drury[1]; Drury and Andrus[2]). This interpretation has been quite generally, but not universally accepted. No human electrocardiograms suggesting the condition have been described so far as we are aware. We have had 2 cases in which the condition is suggested. One case of interference dissociation showed a most unusual phenomenon. There were a large number of auricular impulses which penetrated to the ventricular pacemaker and disturbed its rhythm. Of these nearly 50% were blocked between that point

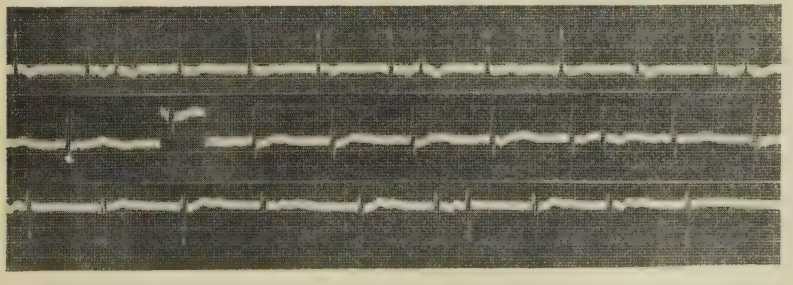

FIG. 1.

Electrocardiograms in leads 1, 2, and 3, showing the A-V rhythm with what appeared to be auricular premature contractions with occasional long R-R intervals of which there were seventeen in all of the tracings. In the T waves of which there is an activity that resembles a blocked premature auricular contraction. This activity, although not reaching the ventricle, apparently succeeds in distinctly prolonging the R-R interval as is shown in the fourth interval in lead 3.

[1] Drury, A. N., *Heart* (London), 1925, **12**, 143.

[2] Drury, A. N., and Andrus, E. C., *Heart* (London), 1924, **11**, 389.

and the ventricle proper. This observation, which appears to be unique, together with further evidence from another case with auricular premature beats, strongly suggests the presence of decremental conduction in the human heart. Even if the tracing be interpreted as one of simple reciprocating rhythm, yet the extreme peculiarity in question remains, namely a descending impulse discharging the ventricular pacemaker yet failing to reach the ventricular musculature.

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Production of Trimethylene Glycol by Fermentation.

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Freund[1] first reported the occurrence of trimethylene glycol in fermentation mixtures while making a study of the production of butyl alcohol. Rayner[2] concluded that the trimethylene glycol must be formed during the spontaneous fermentation of soap lyes subsequently to the liberation of glycerol. He believed that trimethylene glycol was produced by microorganisms. Braak[3] isolated and named *Bacterium freundii* isolated from ditch water, an organism producing trimethylene glycol. He discusses the chemism of the process in some detail.

The present work undertakes to make a systematic study of organisms producing trimethylene glycol. Twelve cultures were isolated from horse, sheep, cow and mouse feces and soils, which produced the glycol from glycerol. All were gram negative short rods occurring in the group generally referred to as intermediate forms of the "coli-aerogenes" group. The 12 cultures were subdivided into 7 species on the basis of fermentative dissimilation of sugars. The group appears to deserve generic ranking. As high as 30% of trimethylene glycol is produced from the fermented glycerol. Typical Escherichia or Aerobacter forms do not produce trimethylene glycol from glycerol. They do produce much greater volumes of CO_2, H_2, ethyl alcohol and succinic acid than do the intermediates, but much less acetic acid.

[1] Freund, A., *Monatsch. Chem.*, 1881, **2**, 636. *Sitzber. K. Akad. Wiss.*, **84**, 671.

[2] Rayner, Archibald, *J. Soc. Chem. Indus.*, 1926, **45**, 265, 287.

[3] Braak, H. R., *Onderzoekingen over Vergisting van Glycerine.* 1928. Thesis, Delft.

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Production of Bacterial Growth Stimulants by Heating the Medium Under Pressure.

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Fulmer and Huesselmann[1] showed the production of yeast growth stimulant by sterilizing sucrose in the presence of ammonium chloride, dipotassium phosphate and a mixture of the 2 salts. Similar results here are recorded for the following bacteria: *Aerobacter faeni, Escherichia freundii,* Actinomyces Sp?, *A. aerogenes, Serratia Marcescens, Esch. coli,* and *Bacillus subtilis.* The first 4 named were considerably more stimulated than the last 3. Treatment of the caramalized media with Norite A removed the coloring matter but not the stimulant. The stimulation took place at any pH value through the viable range. The results are especially interesting in view of the recent report by Lewis[2] that the growth of several bacteria studied by him was inhibited by caramalization of the medium in the presence of nitrogenous compounds.

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Demonstration of Presence of Fowl Pox Virus in Wild Caught Mosquitoes (Culex Pipiens).

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Kligler, Muckenfuss and Rivers[1] have shown that fowl pox could be transmitted experimentally by culex and stegomyia mosquitoes when fed on lesions of fowl pox infected chickens, either by interrupted or successive feeding. They also showed that when culex were liberated in a box containing healthy and infected chickens, the healthy chickens soon developed a fowl pox infection. Kligler

[1] Fulmer, E. I., and Huesselmann, B., *Iowa State Coll. J. Sci.*, 1927, **1**, 411.

[2] Lewis, I. M., *J. Bact.*, 1930, **19**, 423.

[1] Kligler, I. J., Muckenfuss, R. S., and Rivers, T. M., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 128; *J. Exp. Med.*, 1929, **49**, 649.

and Aschner[2] confirmed these findings and showed that the virus remained on the proboscis at least 14 days but could not be demonstrated within the insect.

We have now obtained evidence that *Culex pipiens* taken in the vicinity of chickens infected with fowl pox harbor the virus. In our experiments with fowl pox virus, healthy and infected chickens were always kept in the same room without the occurrence of spontaneous infections. During October spontaneous infections suddenly appeared among our healthy native chickens, kept in separate cages in the same room with infected ones. This room was heavily infested with *Culex pipiens*. The chickens were bought September 14th and the infection appeared between September 25th and 28th.

At the same time, 5 leghorn cockerels kept in an adjacent screened room remained uninfected. One of these was inoculated October 27th and became positive October 30th. November 10th, 10 additional leghorns were put in this room. Three days later mosquitoes were discovered in this room. The 14 healthy chickens were then transferred to another room. All developed spontaneous infections about a week after removal from the mosquito infested room.

At the same time (November 13) mosquitoes were caught in the screened as well as in the large room where the infected chickens were kept. Twenty-six mosquitoes were caught; 7 of these were fed on one wattle, and the heads of the other 19 were rubbed onto the other wattle after scarification. Eight days later a typical infection developed at the point of inoculation, and on the 10th day a vesicle appeared at the point where the mosquitoes were allowed to feed.

On November 16th, 360 mosquitoes were caught in a room near the animal house, where they had apparently collected for hibernation. The heads were triturated and the thick paste inoculated on the wattle of each of 2 chickens. After 7 days both points of inoculation were covered with a typical fowl pox lesion; on one wattle there were 5 discrete vesicles, while on the other there was a massive lesion.

These observations demonstrate that spontaneous infections developed among healthy chickens kept in separate cages in the same room with infected ones. These infections occurred when the room was infested with culex mosquitoes. Some of these mosquitoes taken in the room where the infections occurred, as well as in a room outside but adjacent to the animal house, were shown by feeding

[2] Kligler, I. J., and Aschner, M., *Brit. J. Exp. Path.*, 1929, **10**, 347.

and inoculation to harbor fowl pox virus. The inference is clear, therefore, that *Culex pipiens* may serve in nature as an active agent in the spread of epidemics of fowl pox among chickens.

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Effect of Renal Vessel Ligation and Insulin on Sugar Tolerance of Phloridzinized Dog.

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Dogs were phloridzinized according to the Coolen method and the D:N ration in the urine determined on the day of experiment. In one or 2 instances when the D:N was not determined, the blood sugar was 40 mgm. % or lower. The dogs were then placed under amytal anesthesia and the tolerance curves run. One cc. of 50% pure glucose solution per kg. of body weight was used and was injected intravenously at a uniform rate. When insulin was used it was incorporated in the glucose solution in amount equal to one clinical unit per kg. of body weight.

After ligation of the renal vessels the tolerance curves naturally reached a much higher level than before. The rate of fall of the sugar level for the first hour following the peak of the curve, also, was much greater after ligation than before. Thus, in one experiment, before ligation the rate of fall was 150 mgm. % the first hour, and after ligation it was 270 mgm. % in the same length of time. When insulin was injected with the sugar the same result was obtained, *i. e.*, the rate of fall after ligation was more rapid than before, but the difference was not so great with as without insulin. In one experiment where insulin was given with the sugar by vein, the rate of fall in one hour was 145 mgm. % before ligation, and 195 mgm. % after ligation. It is clear that tying off the renal blood vessels and thereby raising the blood sugar level accelerates the rate of utilization of sugar (combustion or glycogen formation, or both) both with and without extra insulin. The smaller differences between the 2 reactions in the presence of extra insulin indicates a lower threshold of combustion, and elimination of the kidney factor (leakage) does not have so much influence on the rate of utilization.

Control experiments on normal dogs gave an identical tolerance

curve before and after ligation of the renal vessels, both when insulin was used and when it was not used. If insulin exerted any power to correct the action of phloridzin on the kidney, there should have been an effect on the tolerance curve similar to that of ligation of the renal vessels. This effect was not noted at any time, from which it may be inferred that insulin exerts its effect wholly on sugar utilization. If phloridzin acted to any considerable extent on the tissues of the body to diminish the power of utilizing carbohydrate when the blood sugar level is artificially raised, ligation of the renal vessels should not, of itself, cause a greater rate of utilization. Since a greater rate was invariably produced by a higher threshold of sugar, it may be inferred that the action of phloridzin is primarily, and probably entirely, renal.